STIC-ILL

W

438450

From: STIC-Biotech/ChemLib
Sent: Friday, March 28, 2003 6:13 AM
To: STIC-ILL
Subject: FW: Request for 4 Journal Articles (09/744,641)

-----Original Message-----
From: Young, Josephine
Sent: Thursday, March 27, 2003 5:14 PM
To: STIC-Biotech/ChemLib
Subject: Request for 4 Journal Articles (09/744,641)

I would like to request the following 4 articles for case no. 09/744,641.

ACCESSION NUMBER: 82624787 CANCERLIT
DOCUMENT NUMBER: 82624787
TITLE: STUDIES ON THE BIOLOGIC ACTIVITY OF PURINE AND PYRIMIDINE ANALOGS.
AUTHOR: Montgomery J A
CORPORATE SOURCE: Southern Res. Inst., Birmingham, AL, 35255.
SOURCE: Med Res Rev, (1982) 2 (3) 271-308.

ACCESSION NUMBER: 1974:491872 CAPLUS
DOCUMENT NUMBER: 81:91872
TITLE: Synthesis of purine nucleoside 6-sulfonates
AUTHOR(S): Rackwitz, Hans R.; Scheit, Karl H.
CORPORATE SOURCE: Abt. Mol. Biol., Max-Planck-Inst. Biophys. Chem., Goettingen, Fed. Rep. Ger.
SOURCE: Chemische Berichte (1974), 107(7), 2284-94

ACCESSION NUMBER: 87:244312 SCISEARCH
THE GENUINE ARTICLE: G9799
TITLE: AZIRIDINYL PUTRESCINE (AZP) DECREASES PROSTATIC WEIGHT AND SUPPRESSES THE ALPHA-DIFLUOROMETHYLORNITHINE (DFMO) INDUCED INCREASE IN DECARBOXYLATED S-ADENOSYL METHIONINE (DC-SAM)
AUTHOR: HESTON W D W (Reprint); LAUDONE V P; HURYK R; COVEY D F
CORPORATE SOURCE: MEM SLOAN KETTERING CANC CTR, NEW YORK, NY, 10021
SOURCE: PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, (1987) Vol. 28, No. MAR, pp. 258.
DOCUMENT TYPE: Conference; Journal

ACCESSION NUMBER: 74:36171 CONFSCI
DOCUMENT NUMBER: 75024715
TITLE: Reactions of aziridines, beta-lactones & epoxides with adenosine & guanosine.
AUTHOR: Roe, R...
SOURCE: Abstracts, Dec 74; $3.00: Dr. D. W. E. Billups, Dept. of Chemistry, Rice University, Houston, Texas 77001..
Meeting Info.: 30th Southwest Regional Meeting of American Chemical Society (A744053). Houston, Texas. 9-11 Dec 74.
American Chemical Society (Southwest Region).
DOCUMENT TYPE: Conference Article

Thanks for all your help!

Josephine Young
Employee No. 79813
AU 1623
CM1 8D04
Mailbox: CM1 8B19
605-1201

3/31
COMPLETED
4
1.26

36 pages

98. A. DiPasquale and J. McGuire, *Exp. Cell. Res.*, **102**, 264 (1976).
99. J. M. Varga, M. A. Saper, A. B. Lerner and P. Fritsch, *J. Supramol. Struct.* **4**, 45 (1976).
100. V. M. Kriwaczek, A. N. Eberle, M. Müller, and R. Schwyzer, *Helv. Chim. Acta*, **61**, 1232 (1978).
101. K. Medzihradszky, A. Magyar, H. Süli-Vargha, and H. Medzihradszky-Schweiger, Proceedings of the 11th IUPAC International Symposium on Chemistry of Natural Products, Golden Sands, Bulgaria, 1978, p. 282.

Studies on the Biologic Activity of Purine and Pyrimidine Analogs

John A. Montgomery

Southern Research Institute, Birmingham, Alabama 35255

I. INTRODUCTION

The rational design of anticancer agents must ultimately be based on an exploitable biochemical difference between normal host cells and invasive cancer cells. Unfortunately, there is little information on unique characteristics of cancer cells that might be exploitable in the pursuit of new agents. Despite this lack, useful agents such as 5-fluorouracil and 6-mercaptopurine have been developed. In this review I have tried to show how an understanding of the metabolism of the natural purines and pyrimidines and their analogs has allowed us to develop a rather large number of compounds capable of killing cancer cells in culture at physiologically attainable drug levels. Many of these compounds show no selective toxicity and so are inactive *in vivo* at their LD_{10} or maximum tolerated dose against the rodent tumors employed. A significant number, however, have demonstrated good, sometimes curative, activity against murine leukemias such as the commonly employed L1210 and P388 diseases, and some of them have activity against solid tumors also. At the present time at least one, 9-β-D-arabinofuranosyl-2-fluoroadenine, a metabolically stable "second generation" drug, is a candidate for evaluation in man by the National Cancer Institute.

In the sections that follow I have detailed some of the collaborative studies on purine-pyrimidine antimetabolites that have been carried out

Medicinal Research Reviews, Vol. 2, No. 3, 271–308(1982)

CCC 0198-6325/82/030271-28$03.80

at Southern Research Institute over the past twenty years by the Organic Chemistry, Biochemistry, and Chemotherapy Research Departments.

II. 6-THIOPURINES

In the early 1940s the concept that substances chemically related to a metabolite might interfere with the normal function of that metabolite in living cells[1,2] attracted widespread interest among chemists and led to the synthesis and evaluation of analogs of the naturally occurring purines and pyrimidines. Among these were 5-bromouracil,[3] 8-azaguanine,[4] and 6-mercaptopurine (MP).[5] The latter compound (Fig. 1) was found to be moderately active against the murine neoplasm sarcoma 180[6] and highly active against another rodent tumor, adenocarcinoma 755.[7] Preliminary clinical trials in acute leukemia that quickly followed[8] were favorable and led to the acceptance of this agent as a mainstay in the treatment of leukemia.[9]

As a result, a number of laboratories, including mine, began investigations of structural modifications of both MP itself and 6-thioguanine (TG), a compound with very similar activity. An appreciation of the importance of biochemical resistance in treatment failures of initially responsive human neoplasms and the understanding that bacterial[10-12] and neoplastic cells[10,11,13] resistant to either MP or 8-azaguanine lacked the capacity to form nucleotides of these analogs[14,15] led to the synthesis of the ribonucleoside of 6-mercaptopurine (MPR)[16,17] in the hope that this compound would be inhibitory to MP-resistant cells. Although this hope was not realized (Table I),[18] MPR, for reasons not yet understood, did show a greater therapeutic index against adenocarcinoma 755 than 6-MP or any of its other analogs.[19,20] Later we found that a derivative of MPR, namely 6-(methylthio)purine ribonucleoside (MeMPR),[21,22] is toxic to cells resistant to MP,[23] and shows therapeutic synergism with MP in the treatment of L1210 in mice.[24] This combination was also superior to MP alone in the treatment of AML in humans.[25] The explanation of these observations, now clearly evident, lies in the metabolism of MP and its derivatives. MP must be converted to its ribonucleotide (MPRP) by hypoxanthine phosphoribosyltransferase (HPRT), an enzyme which is lacking in cells resistant to the drug.[10-13] MPRP[26,27] is not effective

John A. Montgomery *is Senior Vice President and Director of the Kettering-Meyer Laboratory of Southern Research Institute where for the past 30 years he has carried out research on the synthesis, metabolism, and biologic evaluation of more than twenty nitrogen heterocycles, nucleoside and nucleotide derivatives of certain of these ring systems, carbohydrates, nitrosoureas, organophosphorus compounds, fluorine compounds, and other types of organic compounds. This work has resulted in over 290 publications, eight patents, and a number of anticancer agents, antiviral agents, antiparasitic agents, and insect chemosterilants.*

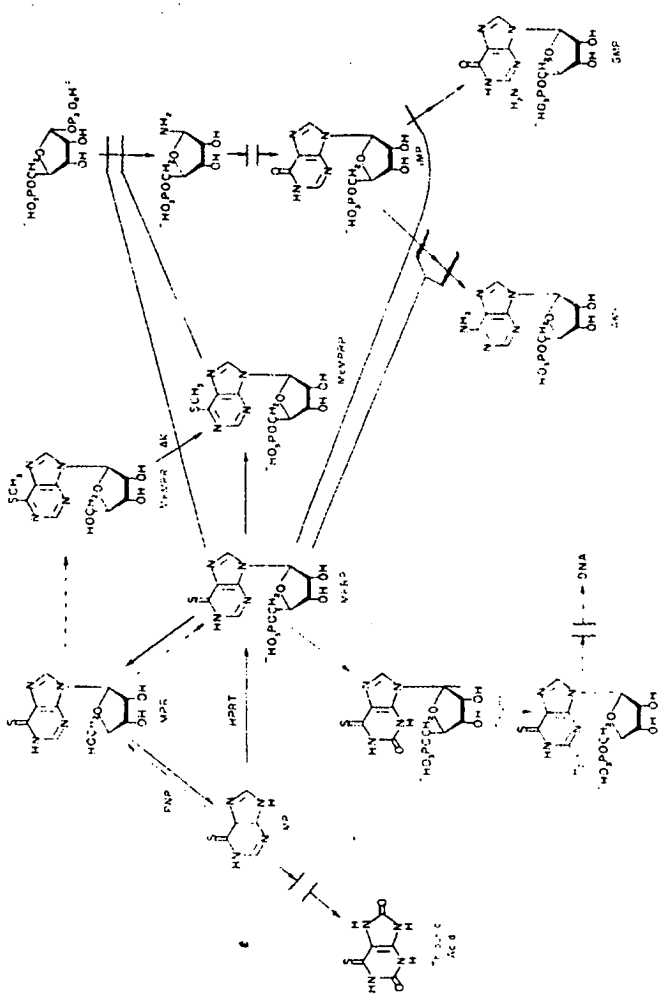


Figure 1. Metabolism of MP and MeMPR and loci of inhibition of their anabolites on the purine nucleotide biosynthetic pathway.

Table I
Cytotoxicity of 6-Thiopurines to H.Ep. No. 2 Cells and a Subline Resistant to 6-Mercaptopurine

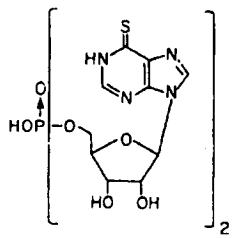
Compound	ID_{50} (μg/ml)[a] H.Ep. No. 2	H.Ep. No. 2/MP[b]	Ratio
6-Mercaptopurine	0.2	400	2000
6-Mercaptopurine ribonucloside	0.4	>100	>250
6-Mercaptopurine ribonucleotide	0.6	>100	>170
6 (Methylthio)purine	>100	>100	--
6-(Methylthio)purine ribonucleoside	0.1	0.1	1
6-(Ethylthio)purine ribonucleoside	0.3	0.3	1
6-(Benzylthio)purine ribonucleoside	3.7	2.8	1.3
6-Thioguanine	0.04	52	1300
6-Thioguanosine	0.4	>100	>250
6-Thioguanylic acid	0.3	>100	>330
S-Methyl-6-thioguanine	44	32	1.4
S-Methyl-6-thioguanosine	>100	>100	--
Bis(thioinosine)-5',5'''-phosphate	0.4	9	23
9-Butyl-6-mercaptopurine	13	8	0.6

[a] The dose required to inhibit the growth of treated cells to 50% that of untreated controls.

[b] Resistant to MP by a deficiency of hypoxanthine phosphoribosyltransferase (HPRT).

because it cannot penetrate cell membranes, but is instead dephosphorylated to MPR. Although MPR is taken up by cells, it is not phosphorylated in most mammalian cell lines, but is cleaved by purine nucleoside phosphorylase (PNP) to MP.[28,29] On the other hand, MeMPR is taken up by cells, is not cleaved by PNP, and is rapidly phosphorylated by adenosine kinase (AK).[23] Other 6-(alkylthio)purine ribonucleosides[17] are phosphorylated less well and cytotoxicity is correlatable with the degree of phosphorylation (Table I).[23] Thus, resistance is circumvented by means of an alternative metabolic pathway, even though the mechanism by which MeMPRP inhibits cellular growth appears to be limited to the blockade of the de novo pathway to inosinic acid by feedback on phosphoribosylpyrophosphate amido-transferase,[30-33] whereas MP inhibits a number of other enzymes also,[34,35] as well as being incorporated into DNA.[36,37] Furthermore, MPRP is methylated in cells to MeMPRP, which presumably contributes to its action.[38] MP and MeMPR are therapeutically synergistic against L1210 but not against resistant cell lines that do not produce both nucleotides.[24] It seems likely that this potentiation is a result of the combined action of the two agents against a heterogeneous cell population containing mutants resistant to the single agents.[30]

Another approach to overcoming resistance was less successful. Since, as mentioned above, intact nucleotides do not enter cells to any

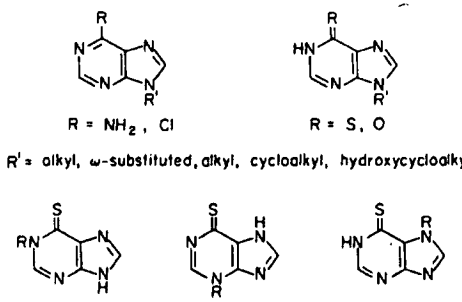


Bis(thioinosine)
5',5'''-Phosphate

Figure 2. Bis(thioinosine) 5',5'''-phosphate.

significant extent, precluding the use of MPRP, MeMPRP, or 8-azaguanylic acid against resistant lines, we prepared a variety of ester derivatives of MPRP and other fraudulent nucleotides[40-43] in the hope that they might penetrate cells and be cleaved to nucleotides intracellularly. Of these, bis(thioinosine) 5', 5'''-phosphate (Fig. 2 and Table I) was quite cytotoxic to cultured cells resistant to MP but is probably rapidly cleaved in serum *in vivo*.[44]

Based on the understanding that MP must be activated by conversion to its nucleotide, we undertook the synthesis of other 9-substituted derivatives that might function in some ways like nucleosides or nucleotides.[45-47] A series of 9-alkylpurines (Fig. 3) proved to be cytotoxic to Human Epidermoid Carcinoma No. 2 (H.Ep.-2) cells and also to an MP-resistant subline (Table 1).[48] Of these the MP derivatives were the most active and some of these were active against adenocarcinoma 755 *in vivo*. 9-Ethyl-6-mercaptopurine was chosen for clinical trial and shown to be about as active as MP in a limited trial against chronic leukemia.[49] Studies on the mechanism if action of the 9-alkylpurines have not been productive, but it is clear that they act differently from MP because they are not converted to it or otherwise significantly metabolized *in vivo*, and they are toxic to MP-resistant cells. In an effort to shed some light on this problem, we prepared 1-, 3-, and 7-alkyl derivatives of MP for com-



R = NH_2, Cl R = S, O

R' = alkyl, ω-substituted alkyl, cycloalkyl, hydroxycycloalkyl

Figure 3. N-Alkylpurines.

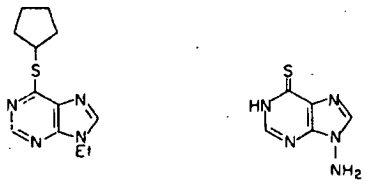


Figure 4. 6-(Cyclopentylthio)-9-ethylpurine and 9-amino-6-mercaptopurine.

parison with the 9-alkyl compounds (Fig. 3).[50,51] Somewhat surprisingly the 1- and 3-alkyl derivatives are as cytotoxic as the 9-isomers whereas the 7-isomers are somewhat less active, being about one-tenth as cytotoxic.[51,52] The fact that all these N-alkylpurines are cytotoxic is compatible with the idea that their activity could be due to binding to nucleic acids in the manner of caffeine rather than the ability of the 9-alkylpurines to mimic purine nucleosides.[51] *In vivo* activity against L1210 leukemia was observed.

Several S,9-dialkyl derivatives of MP designed for lipid solubility were synthesized and evaluated against adenocarcinoma 755 implanted both subcutaneously and intracerebrally and against L1210 leukemia implanted both ways to assess their ability to cross the blood–brain barrier and kill cancer cells.[53] One compound, 6-(cyclopentylthio)-9-ethylpurine (Fig. 4) appeared to be more effective than MP against these intracerebral diseases.

Among other 9-substituted purines 9-amino-6-mercaptopurine (Fig. 4) and some of its derivatives showed activity in the L1210 system comparable to MP and may owe their activity to *in vivo* conversion to MP.[54,55] Attempts to extend these observations to the preparation of purines with antimalarial activity were unsuccessful.[56,57]

III. AZAPURINES

Interest in the azapurines dates back to the discovery of the antibacterial and antitumor activity of 8-azaguanine (8-azaG) (Fig. 5).[58] The closely related 8-azahypoxanthine (8-AzaH) was later shown to possess moderate activity against adenocarcinoma 755, but was not active against L1210.[20] Its ribonucleoside, 8-azainosine (8-azaHR),[59–61] however, proved to be active not only against Ca 755 and L1210, but also against L1210/MP, a strain of this leukemia deficient in HPRT and, as a consequence, cross resistant to 8-azaH.[62] These results prompted further studies on this interesting nucleoside as well as on a variety of other 2- and 8-azapurine nucleosides.[63,64]

The activity of 8-azaHR against cells resistant to 8-azaH is good evidence that conversion to 8-azaH is not a required step in its activation. In contrast, these resistant cells, which are also resistant to the 6-thiopurines, are resistant to the corresponding 6-thiopurine nucleosides which apparently, in intact cells, are converted to nucleotides only after

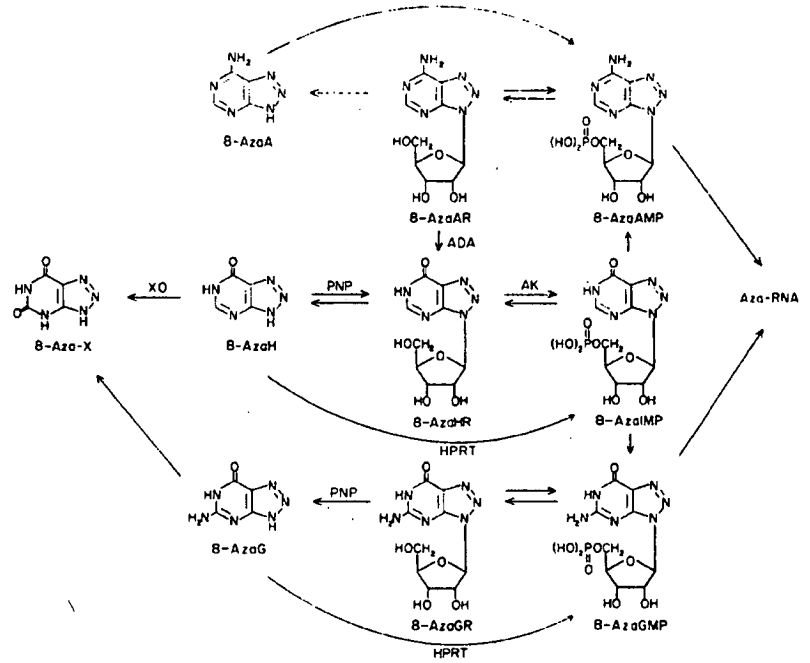


Figure 5. Metabolism of the 8-azapurines.

cleavage to the free bases. Likewise these lines are 200 to 500-fold resistant to 8-azaG and 20-40-fold resistant to 8-azaguanosine (8-azaGR) (Table II).[64] An H. Ep.-2 mutant subline resistant to both MP and MeMPR and deficient in HPRT and AK was 40–80-fold resistant to 8-azaHR, a result consistent with the fact that 8-azaHR is a substrate albeit a poor one, for AK from these cells.[65] Thus 8-azaHR must be converted to its monophosphate to exert its biologic activity and that conversion may occur either by direct phosphorylation or via 8-azaH. In the case of 8-azaGR direct phosphorylation must occur also, at least in cell lines deficient in HPRT, but probably by a different kinase and less efficiently than with 8-azaHR. Other nucleosides of 8-azaH are less cytotoxic and devoid of activity against L1210 leukemia *in vivo*.[63,64]

Further studies[66] compared the activity and metabolism of 8-azaadenosine (8-azaAR)[59,67] and 8-azaHR. These studies indicate that 8-azaHR is incorporated into polynucleotides as 8-azaG and that this incorporation is responsible for its cytotoxicity. In H. Ep.-2 cells 8-azaHR is incorporated to about one-half the amount found when 8-azaGR was the precursor. 8-AzaAR is incorporated about equally as 8-azaadenine (8-azaA) and 8-azaGR. In cells lacking adenine phosphoribosyl transferase (APRT) and AK, 8-azaAR and 8-azaHR are metabolized similarly in that

Table II
Cytotoxicity, H. Ep. No. 2 Cells in Culture

Compound	ID_{50}, μM[a]	Degree of Resistance[b] HPRT⁻[c]	APRT⁻[d]	AK⁻[e]	APRT⁻/AK⁻[f]
8-Azaadenine	20	1-2		2	>10
8-Azaadenosine	0.8	70		2-3	2-3
8-Azahypoxanthine (8-AzaH)	3	>300	1	1	1
8-Azainosine (8-AzaHR)	0.7	1-2	1	1	1
8-Azaguanine (8-AzaG)	3	200	1	1	1
8-Azaguanosine (8-AzaGr)	2	20-40	1	1	1
8-Aza-6-mercaptopurine (8-AzaMP)	>300				
8-Aza-8-Thioinosine (8-AzaMPR)	(1)	1		90	40
[1,2,3]Thiadiazolo[5,4-d]pyrimidin-7-amine (TP)	>70				
N-β-D-Ribofuranosyl[1,2,3]thiadiazolo-[5,4-d]pyrimidin-7-amine (TPR)	0.1				
8-Aza-6-thioguanine (8-AzaTG)	4				
8-Aza-6-thioguanosine (8-AzaTGR)					
[1,2,3]Thiadiazolo[5,4-d]pyrimidin-5,7-diamine	>300				
N^7-β-D-Ribofuranosyl[1,2,3]thiadiazolo-[5,4-d]pyrimidin-5,7-diamine	>300				
8-Aza-6-(methylthio)purine	>300				
8-Aza-6-(methylthio)purine ribonucleoside	0.02			2000	2000
8-Aza-6-(ethylthio)purine ribonucleoside	0.1				300
8-Aza-O^6-methylinosine	0.3			6	6
8-Aza-O^6-ethylinosine	0.1			20	
8-Aza-O^6-methylguanosine	2				
8-Aza-8-methyl-6-thioguanosine	>100				
8-Aza-5-methyl-6-thioguanosine	>100				

[a] Dose required to inhibit growth of treated cells to 50% of controls.
[b] Ratio of ID_{50} for resistant cells to ID_{50} for sensitive cells.
[c] Resistant to MP-deficient in hypoxanthine phosphoribosyltransferase.
[d] Resistant to 2-fluoroadenine-deficient in adenine phosphoribosyltransferase.
[e] Resistant to 6-(methylthio)purine ribonucleoside-deficient in adenosine kinase.
[f] Resistant to 2-fluoroadenine and 2-fluoroadenosine-deficient in adenine phosphoribosyl transferase and adenosine kinase.

both are incorporated into polynucleotides principally as 8-azaG, indicating that in these cells 8-azaAR, a better substrate for adenosine deaminase (ADA) than adenosine itself, is metabolized by conversion to 8-azaHR. A cell line resistant to 8-azaHR but sensitive to 8-azaAR, and which has HPRT activity, metabolized 8-azaHR to only a small extent; 8-azaAR was more extensively metabolized and was incorporated primarily as 8-azaA. The failure of these cells to convert 8-azaAR or 8-azaHR to 8-azaG nucleotides indicates that resistance may be due to a change in the substrate specificities of the enzymes of guanylic acid (GMP) synthesis such that these cells cannot convert 8-azainosinic acid (8-aza-IMP) to 8-aza-GMP. 8-AzaAR is a potent inhibitor of purine nucleotide synthesis de novo but 8-azaHR is not. Thus the effects of 8-azaAR are more complex than those of 8-azaHR to which it is normally, at least in part, converted.

As detailed in Sec. II, MPR was ineffective against cells resistant to MP

because it is not phosphorylated and is rapidly cleaved to MP. The finding that 8-azaHR is a relatively poor substrate for PNP and is phosphorylated by AK led to the prediction that 8-aza-6-thioinosine (8-azaMPR) (Fig. 6) would be a good substrate for AK and not PNP and therefore show activity against MP-resistant cells.

Pure 8-azaMPR can be isolated as its sodium salt, but the apparent instability of the free nucleoside led to studies resulting in the observation that heating dry 8-azaMPR at 138° C converts it quantitatively to N-β-D-ribofuranosyl [1,2,3] thiadiazolo [5,4-*d*] pyrimidin-7-amine (TPR), identified by spectral data and elemental analyses.[68,69] Since this rearrangement also occurs in aqueous media at pH 7.4 and room temperature, interpretation of the observed biologic activity of azaMPR required a detailed study of the rearrangement and an investigation of the activity of TPR, which is not converted back to azaMPR under these conditions in six days (at 100° C the reverse rearrangement does take place—in two minutes about 30% conversion of TPR to azaMPR was observed). First, we found that the rearrangement to TPR is pH-dependent; thus, as might be expected, the anion of 8-azaMPR appears to be incapable of undergoing rearrangement. Although, as stated above, rearrangement

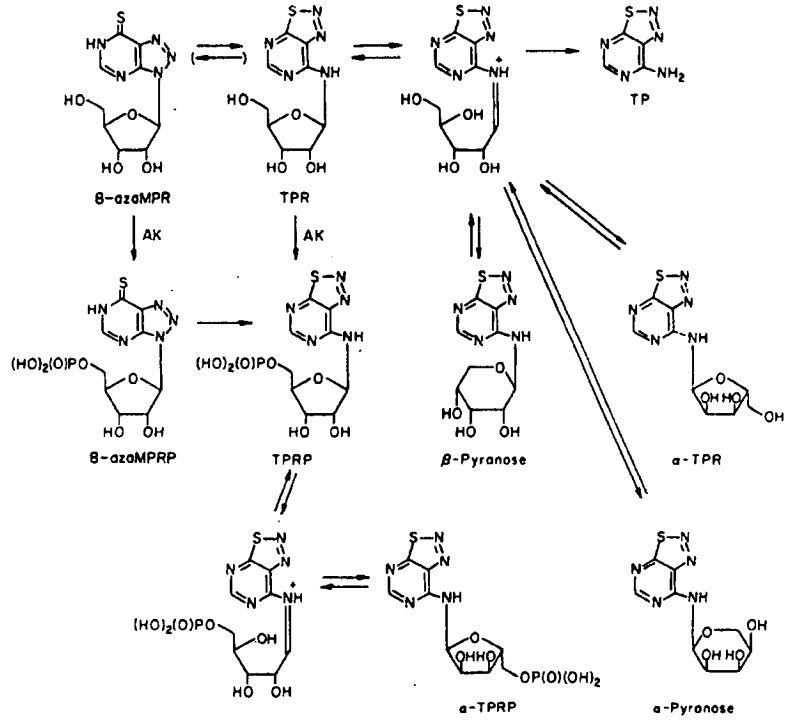


Figure 6. Chemical and enzymatic transformations of 8-aza-6-thioinosine.

back to azaMPR does not occur at room temperature, a different rearrangement does take place—the ribofuranose ring opens and recloses giving rise to three new nucleosides in roughly equal amounts: the α-ribofuranose and the α- and β-ribopyranoses (Fig. 6). TPR and its isomers can also break down to [1,2,3] thiadiazolo [5,4-*d*] pyrimidin-7-amine (TP), an inactive compound (Table II). The chemistry described for TPR and its isomers is typical of glycosylamines and similar to that observed for ribosylaminopyrimidines.[70]

AzaMPR and TPR both inhibited the growth of H. Ep.-2 cells in culture, but TPR was tenfold more cytotoxic (Table II). These results suggested that both of these compounds are phosphorylated, perhaps by AK, and this was shown to be the case; in fact, both azaMPR and TPR are phosphorylated more rapidly than adenosine. Since 8-azaMPR rearranges, under the conditions of the cytotoxicity tests, to TPR, which is an order of magnitude more cytotoxic than 8-azaMPR and is phosphorylated equally rapidly, TPR could be responsible for the activity of 8-azaMPR. The reverse seemed unlikely to be true since the rearrangement of TPR to 8-azaMPR could not be detected under the conditions of the cytotoxicity tests. The metabolism of 8-azaMPR and TPR was then investigated in H. Ep.-2 cells: 8-azaMPR gave three nucleotides, two of which were identified as thiadiazolo [4,5-*d*] pyrimidines and the third one as an 8-azaMP; the thiadiazolo [4,5-*d*] pyrimidines must be the α- and β-D-ribofuranoses (Fig. 6).[71,72] Rearrangement of the 8-azapurine ring could have occurred before or after phosphorylation, and rearrangement of the sugar moiety could have occurred prior to phosphorylation, but any pyranoses formed could not then be phosphorylated. Further, studies on the substrate specificity of adenosine kinase would suggest that the α-ribonucleotide observed probably resulted from rearrangement of the β-ribonucleotide (TPRP). In any event, these results indicate that it is not possible to separate the activity of azaMPR from that of its rearrangement product TPR. On the other hand, similar experiments with TPR showed the formation of only two nucleotides—TPRP and its α-anomer. That the β-anomer is responsible for cytotoxicity is indicated by the cytotoxicity (KB cells) of an approximately equimolar mixture of the four ribose derivatives, which can be accounted for by the amount of β-anomer present. There is no evidence for cleavage of 8-azaMPR by PNP or for hydroxydethiolation by ADA. In H. Ep.-2 cells there is no indication of any conversion to 8-azaGR triphosphate, indicating that hydroxydethiolation does not occur in whole cells by any other enzyme either. Thus, the activity of these thio compounds (8-azaMPR and TPR) is unrelated to 8-azaHR. Both 8-azaMPR and TPR selectively reduce the pools of guanine nucleotides and inhibit the synthesis of RNA and DNA without affecting protein synthesis. The inhibition of growth of H. Ep.-2 cells by both agents is partially reversed by hypoxanthine and 4-aminoimidazole-5-carboxamide. In addition, adenine and uridine give

some reversal of TPR. Guanosine potentiates the action of both inhibitors, as it does for other adenosine analogs. Thus, although the chemistry and metabolism of these thio analogs of purines have been elucidated (Fig. 6), there is, as yet, no evidence for the primary site of action of either compound or for a decision as to the true role of 8-azaMPRP.

An extension of this study to 8-aza-6-thioguanosine (8-azaTG) gave quite different results.[73] Although 8-azaTG is about as cytotoxic as 8-azaG, the ribonucleoside of the thio compound was inactive as were the thiadiazolo [5,4-*d*] pyrimidines derived from both thio compounds (Table II). It would appear that neither nucleoside is phorphorylated, but that 8-azaTG is probably a substrate for HPRT. Substitution of other groups for the sugar moiety of 8-azaMPR was equally unproductive. Thus, 9-α-D-arabinofuranosyl-8-aza-6-mercaptopurine and its rearrangement product—compounds having the proper stereochemistry to be substrates for AK—and 8-aza-9-butyl-6-mercaptopurine and its rearrangement product were all inactive against KB cells in culture. Indeed, although TPR represents an interesting new structural variant that is phosphorylated to a cytotoxic nucleotide with activity against leukemia L1210 *in vivo*, these findings may be somewhat limited in scope.

The synthetic intermediate for the preparation of 8-azaMPR, 8-aza-*S*-methyl-6-thioinosine (Fig. 7), is an aza analog of MeMPR and of considerable interest in its own right.[69] It is one of the most cytotoxic synthetic nucleosides prepared to date, being 50 times as cytotoxic as the corresponding purine ribonucleoside and equal in potency to 2-fluoroadenosine (Table II). It is an extremely poor substrate for ADA and an

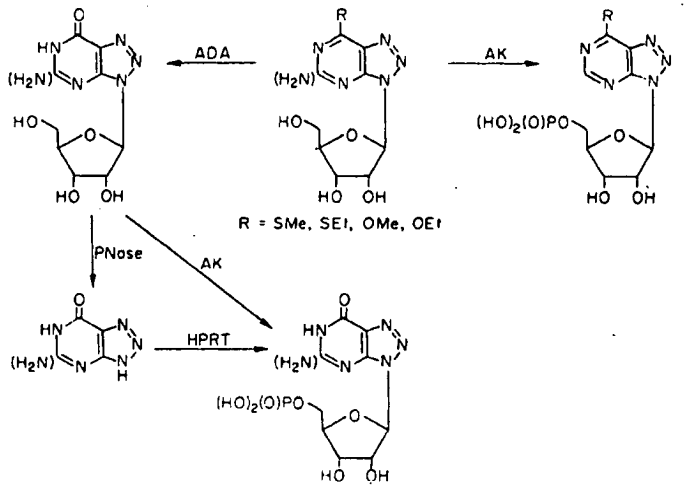


Figure 7. Alternative routes of metabolism of the S-alkyl and O^6-alkyl derivatives of 8-azapurines.

excellent substrate for adenosine kinase; and cells lacking adenosine kinase are resistant to it (Table II), so that this analog also appears not to owe its activity to conversion to 8-azaHR, and hence to incorporation into nucleic acids. The ethyl analog is one-fifth as active as the methyl compound, probably because it is not phosphorylated as well by the kinase. All these results in the 8-azapurine series are in keeping with previous results with the 6-(alkylthio)purine ribonucleosides.[22,23]

The closely related O^6-methyl derivative of 8-azaHR (Fig. 7) is more complex, because it is a good substrate for both the kinase and the deaminase (Table III) so that its activity may depend on its conversion to either 8-aza-O^6-methylinosinic acid, which should resemble the methylthio compound, or to 8-azainosinic acid, which is quite different.[70,74] Indeed, cell lines lacking adenosine kinase are somewhat resistant to both the O^6-methyl and the O^6-ethyl compounds, but nowhere near as resistant as they are to the alkylthio compounds (Table II), indicating that conversion of 8-azaHR does contribute to the toxicity of those compounds. At the same time, the low level of resistance indicates the contribution of the O^6-alkyl compounds. Three results led us to examine the effect of 2'-deoxycoformycin (DCF), a potent inhibitor of adenosine deaminase, on the cytotoxicity of these compounds (Table IV). Partial reversal of the activity of the O^6-methyl compound was observed consistent with the previous results. On the other hand, DCF had little effect on the activity of the O^6-ethyl compound, a relatively poor substrate for ADA. Most striking was the essentially complete reversal of the toxicity of 8-aza-O^6-methylguanosine by DCF. 8-Aza-O^6-methylguanosine is an excellent substrate for the deaminase, and consistent with the other results with guanine analogs, is not a substrate for

Table III
Kinetic Constants for the Action of Adenosine Deaminase and Adenosine Kinase on Adenosine and Analogs

Compound	Adenosine Deaminase		Adenosine Kinase	
	K_m (μM)	V_{max} (nmoles/min/mg)	K_m (μM)	V_{max} (μmoles/min/mg)
Adenosine	29	435	1.8	222
6-(Methylthio)purine ribonucleoside			50	733
8-Aza-8-thioinosine (azaMPR)			210	333
N- -D-Ribofuranosyl[1,2,3]thiadiazolo-[5,4-d]pyrimidine-7-amine (TPR)			140	266
8-Aza-S-methyl-6-thioinosine	--*		85	333
O^6-Methylinosine	50	2	91	284
8-Aza-O^6-methylinosine	140	27	240	115
8-Aza-O^6-ethylinosine	360	0.7	190	31
8-Aza-O^6-methylguanosine	22	20		

*Conversion of the substrate to 8-azainosine occurred when it was incubated for 24 h in the presence of a large excess of ADA, but the rate was too slow for convenient determination of kinetic constants.

Table IV
Effects of 2'-Deoxycoformycin (DCF) on the Cytotoxicity to H.Ep.-2 Cells of Some O^6-Alkyl-8-Azainosines and O^6-Methyl-8-Azaguanosine

Compounds (μM)	Colony Formation: % of Control
DCF (3.7)	99
O^6-Methyl-8-azainosine (0.18)	26
O^6-Methyl-8-azainosine (0.35)	5
O^6-Ethyl-8-azainosine (0.17)	4
O^6-Methyl-8-azaguanosine (3.4)	22
O^6-Methyl-8-azainosine (0.18) + DCF (3.7)	70
O^6-Methyl-8-azainosine (0.35) + DCF (3.7)	20
O^6-Ethyl-8-azainosine (0.17) + DCF (3.7)	6
O^6-Methyl-8-azaguanosine (3.4) + DCF (3.7)	96

adenosine kinase. 8-Aza-S-methyl-6-thioguanosine does not appear to be a substrate for any cellular deaminase or kinase since it is not cytotoxic.

In a way, these results are not surprising since O^6-methylinosine is known to be a substrate for ADA. At the same time, the progression of the K_m and V_{max} values in Table III is certainly at variance with the commonly accepted rule in antimetabolite design that one change in the structure of a metabolite may produce an antimetabolite, but a second change will destroy the desired activity. In this case, one change produced O^6-methylinosine with a twofold increase in K_m and a greatly reduced V_{max}. A second change produced 8-aza-O^6-methylinosine with a still higher K_m, but with a 15-fold increase in V_{max} over O^6-methylinosine. And yet another change, three in all, produced 8-aza-O^6-methylguanosine with a K_m lower than that of adenosine and a V_{max} almost as great as that of 8-aza-O^6-methylinosine. Another interesting aspect of this work is the development of an analog that is activated by a catabolic enzyme. Inhibition of that enzyme by DCF, which enhances the activity of many adenosine analogs, completely destroys the activity of 8-aza-O^6-methylguanosine.

Our observations on the antitumor activity of 8-aza-HR and on the cytotoxicity of 8-azaAR, 8-azaHR, and some related thio, alkylthio, and alkyloxy compounds led us to investigate the nucleosides of 2-azapurines.[63,64,75-77] The bases, 2-azaadenine (2-azaA) and 2-azahypoxanthine (2-azaH) (Fig. 8) had long been known to inhibit the growth of both microbial and mammalian cells, but the nucleosides had not been prepared.

2-Azaadenosine (2-azaAR) is much more toxic than 2-azaA (Table V) and is a good substrate for both AK and ADA.[64,78] The responses of resistant cell lines indicated that the cytotoxicty of 2-azaAR is due both to its direct phosphorylation and to its conversion to 2-azaH. 2-AzaHR,

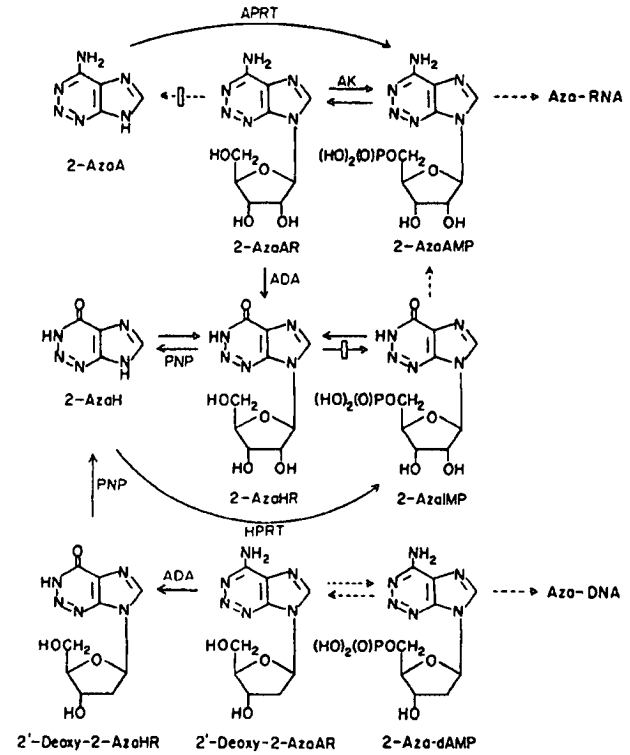


Figure 8. Metabolism of the 2-azapurines.

2′-deoxy-2-azaHR, and 2-azaH had similar cytotoxicities, and the responses of the resistant cell lines showed that the activity of both nucleosides resulted from their conversion to 2-azaH. The ribonucleoside of 4-aminopyrazolo [3,4-*d*]-v-triazine is less toxic than 2-azaAR even though it is a good substrate for AK and a poor substrate for ADA. 2-AzaHR and 2′-deoxy-2-azaHR are cleaved to 2-azaH by cell-free supernatants, but the conversions were poor relative to those of inosine and 2′-deoxyinosine. 3′-Deoxy-2-azaHR and the α-arabinofuranosyl, β-arabinofuranosyl, and β-xylofuranosyl derivatives of 2-azaA were not toxic. 3′-Deoxy-2-azaAR and 9-β-D-xylofuranosyl-2-azaA were moderately good substrates for ADA, but under the conditions of the experiment 9-α-D-arabinofuranosyl-2-azaA was not deaminated and the β-anomer was deaminated at a rate less than five per cent that of 9-β-D-arabinofuranosyladenine (ara-A). Of these nucleosides only 2-azaAR has shown modest *in vivo* activity against leukemia L1210.[64]

2-AzaAR and its relatives are prepared from the corresponding adenine nucleosides by ring opening and reclosure with nitrous acid. The antiviral activity of one of the intermediates in this sequence, 1-

Table V
Cytotoxicity and Substrate Specificity of Some 2-Azapurines

Compound	ID_{50} (μM) H.Ep. No. 2 Cells	Degree of Resistance[a] HPRT[-][b]	APRT[-][c]	AK[-][d]	HPRT[-]/APRT[-][e]	APRT[-]/AK[-][f]	HPRT[+]/AK[-][g]	Ado Kinase Relative Activity[h]	Ado Kinase K_m (μM)	Ado Deaminase Relative Activity[h]	Ado Deaminase K_m (μM)	PNP Relative Activity[i]
2-Azahypoxanthine	0.4	600	2	1	600	2	600					
2-Azainosine	0.9	150	0.8	0.6	150	0.4	150					8
2′-Deoxy-2-azainosine	0.6	200		1								6
3′-Deoxy-2-azainosine	60											
9-β-D-Arabinofuranosyl-2-azahypoxanthine	40-80											
7-β-D-Ribofuranosylpyrazolo[3,4-d]-v-triazin-4-one	75											
2-Azaadenine	11	1	30	2	30	30	3					
2-Azaadenosine	0.2	0.7	1	2	0.9	3	600	370	200	102	670	
2′-Deoxy-2-azaadenosine	7									230	1300	
3′-Deoxy-2-azaadenosine	75									14	500	
9-β-D-Arabinofuranosyl-2-azaadenine	75									0.8	260	
9-β-D-Xylofuranosyl-2-azaadenine	75									49	1250	
4-Amino-7-β-D-ribofuranosylpyrazolo[3,4-d]-v-triazine	15							92		0.2	440	

[a] Ration of ID_{50} H. Ep. No. 2/O to ID_{50} H. Ep. No. 2/R.
[b] Resistant to MP.
[c] Resistant to 2-Fluoroadenine.
[d] Resistant to MeMPR.
[e] Resistant to MP and 2-Fluoroadenine.
[f] Resistant to 2-Fluoroadenine and 2-Fluoroadenosine.
[g] Resistant to MP and MeMPR.
[h] Adenosine = 100, K_m for AK = 1.8, for ADA = 29.
[i] Inosine = 100, 2′-deoxyinosine = 60.

(benzyloxy)adenosine, led to the synthesis of a series of analogs substituted on the phenyl ring and optimization of this activity.[79]

IV. α-ARABINONUCLEOSIDES

Many adenosine analogs have biological activity, but only a few have sufficient specificity to be considered effective antitumor agents. The limitation on the activity of many adenosine analogs is their susceptibility to the action of ADA because the resulting inosine analog is usually inactive or less active. One of the most interesting adenosine analogs is β-araA. β-AraA has been known for some years to have some antitumor activity and to be a potent inhibitor of DNA viruses.[80] Its antitumor activity is limited by its facile deamination, and it has proven to be a useful antitumor agent only in the last few years after potent inhibitors of ADA became available for use in combination with it.[81,82]

Because of the many adenosine analogs that are known and because of the extensive studies with ADA, it seemed that it should be possible to design an analog of β-araA that would not be deaminated and would yet have the inhibitory properties of β-araA. One type of nucleoside that at first appeared to show promise was a by-product of our synthesis of some β-araA analogs in which the purine ring was modified.[61] One of the compounds prepared was β-ara-8-azaadenine which turned out to be without interesting biological activity. A by-product of its preparation was the α-anomer (Fig. 9),[83] which surprisingly had considerable cytotoxicity.[84] This was unexpected because α-anomers generally have been considered to be biologically inert. However, it was known that α-adenosine and α-2′-deoxyadenosine were not substrates for adenosine

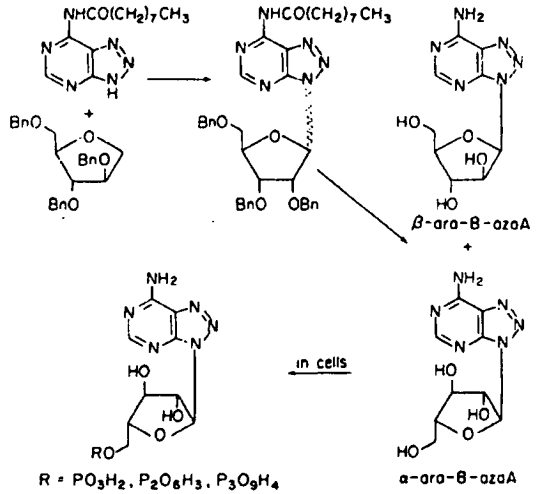


Figure 9. Synthesis and metabolism of 9-α-D-arabinofuranosyl-8-azaadenine.

deaminase and it appeared then that the resistance to deamination might be a factor in the growth-inhibitory activity of α-ara-8-azaA. Our interest was further heightened by the observation that its *in vitro* antiviral activity was equal to that of β-araA. α-AraA also was found to be cytotoxic and to have high *in vitro* antiviral activity. Further investigation of α-ara-8-azaA showed that, as expected, it is not deaminated; in cell cultures it is converted to mono-; di-, and triphosphates and the kinase catalyzing its initial phosphorylation is adenosine kinase. The remaining question was whether the triphosphates of α-araA and α-ara-8-azaA had the same metabolic effects as β-araA. For this study, we chose α-araA so that a direct comparison could be made between α- and β-anomers of the same nucleoside. α-AraATP was prepared and compared with β-araATP with respect to inhibition of DNA polymerase, which is the primary site of action of β-araATP, and to inhibition of ribonucleotide reductase, which is also inhibited by β-araATP at concentrations that can be attained *in vivo*.[85] α-AraATP, at concentrations up to $10^{-3}M$ failed to inhibit the DNA polymerase activity of a preparation from H. Ep.-2 cells, whereas β-araATP inhibited at concentrations of 10^{-5}–$10^{-4}M$, as was expected from earlier reports. For measurements of effects on the reductase, we used a preparation from cultured L1210 cells. β-AraATP at a concentration of $5 \times 10^{-5}M$ inhibited the reduction of 2′-deoxycytidine diphosphate by 50%, whereas α-araATP produced no inhibition at concentrations up through $1 \times 10^{-3}M$. Neither α-araA nor α-ara-8-azaA have been found to have activity against L1210 leukemia. Thus, although these β-arabinosyl compounds are metabolized in the desired fashion, they cannot be considered as analogs of β-araA insofar as mechanism of action is concerned. A number of additional α-D-arabinonucleosides have been prepared from purines, azapurines, and pyrimidines, but only one, the α-anomer of 1-β-D-arabinofuranosylcytosine, showed any biologic activity: it was toxic to H. Ep.-2 cells in culture (ED_{50} = 10 μg/mL), but showed no activity against L1210 leukemia *in vivo*.[86] It did give a significant, dose-dependent inhibition of Gross Mouse Leukemia Virus in mouse embryo cells at less than one-tenth the cytotoxic level, indicating a high degree of antiviral selectivity *in vitro*.

Studies with the α-arabinonucleosides and other purine nucleosides have led to the conclusion that necessary, but not sufficient, conditions for good substrate activity for adenosine kinase are: that the purine or purine analog nucleoside have a five-membered ring containing a 2′-hydroxyl group trans to the heterocyclic ring and a 4′-hydroxymethyl group and that there exist a considerable degree of freedom of rotation about the C-1′-N-9 bond. If these conditions are met, the 3′-hydroxyl group, which is not required, and the 4′-hydroxymethyl may be either cis or trans to the heterocyclic ring.[87] This conclusion, which as been supported by others,[88] should aid in the design of new nucleoside analogs as growth-inhibiting or antitumor agents.

V. CARBOCYCLIC ANALOGS OF NUCLEOSIDES

As discussed above, phosphorolytic cleavage of nucleosides by enzymes such as 6-thioinosine (MPR) by PNP can negate their potential utility as chemotherapeutic agents (Sec. I). The concept of enzymatically stable nucleoside analogs and the biologic activity of the 9-cycloalkyl purines[48,51] led to the synthesis of carbocyclic (cyclopentane) analogs of nucleosides in which the furanose oxygen atom is replaced with a methylene group and the hydroxyl groups occupy the same positions, having the same cis-trans relationships and assume similar conformations. Such analogs have the potential to mimic or antagonize the function of the naturally occurring nucleosides and, after phosphorylation, nucleotides; but unlike nucleosides, these analogs have a carbon-nitrogen bond joining the heterocyclic base to the cyclopentane ring comparable in stability to that of a simple alkyl derivative (see above) and, therefore, not susceptible to enzymatic fission as is the glycosyl bond of true nucleosides.

The first compound of this type prepared, the racemic carbocyclic analog of adenosine (C-Ado) (Fig. 10), proved to be biologically active as expected.[89-91] It is highly cytotoxic to both H. Ep.-2 and L1210 cells in culture, but was not effective against L1210 leukemia *in vivo* at the maximum tolerated dose. Its cytotoxicity is increased by guanine, the utilization of which it strongly inhibits.[92] The effect on guanine metabolism is reflected by a decreased incorporation of guanine into nucleic acids and a decreased accumulation of guanine metabolites in the alcohol-soluble fraction of cells. It also causes an accumulation of xanthosine, reduces the AMP to IMP ratio in cells, and inhibits an early step of de novo purine nucleotide biosynthesis. C-Ado monophosphate is a potent competitive inhibitor of GMP kinase but does not inhibit HPRT or GDP kinase. Inhibition of GMP kinase may be responsible for the growth-inhibiting properties of C-Ado and may be involved in the increased toxicity of the analog in the presence of guanine, since it is rapidly converted to the mono-, di-, and triphosphates, and to some extent, deaminated in intact cells. No compound migrating like nicotinamide-adenine dinucleotide (NAD) was detected and little or no incorporation of its nucleic acids occurred.[93] C-Ado is a substrate for AK (from H. Ep.-2 cells) and ADA (from calf intestine). Cell lines lacking AK have at best a low level of resistance to C-Ado, indicating that the nucleoside analog itself has potent growth-inhibitory properties. The recent finding that this compound is both a potent reversible inhibitor[94] and irreversible inactivator[95] of adenosylhomocysteinase may explain the activity of C-Ado itself, since inhibition of this enzyme is known to cause an accumulation of adenosylhomocysteine which interferes, by feedback, with vital transmethylation reactions involving adenosylmethionine (see later).[96]

The carbocyclic analogs of 2'- and 3'deoxyadenosine,[97] inosine,[98] and guanosine[99] are all devoid of cytotoxicity, presumably because they are

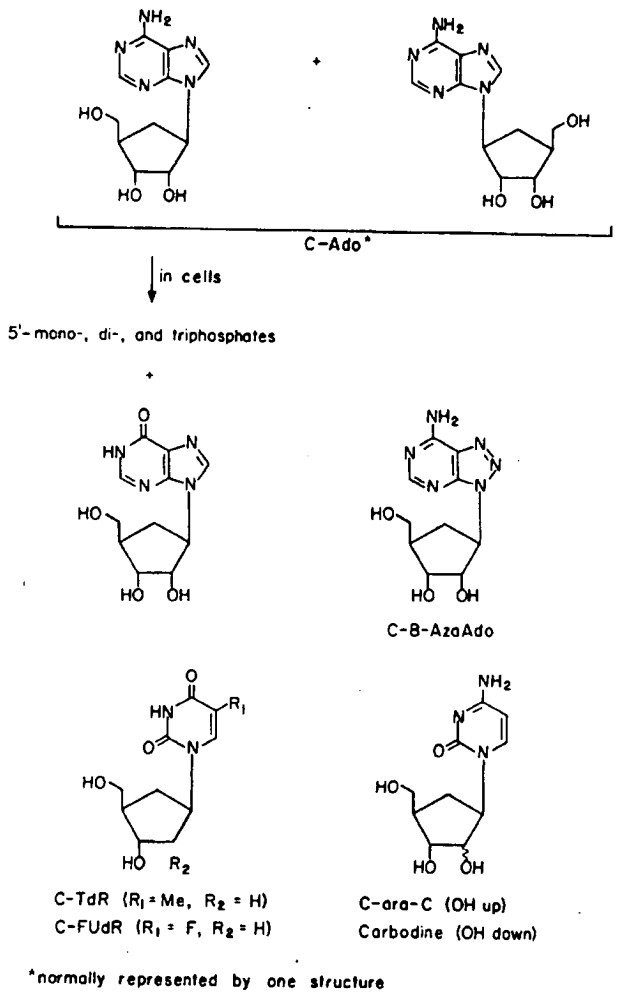


Figure 10. The carbocyclic analogs of purine and pyrimidine nucleosides.

not phosphorylated in cells, although the guanosine analog proved to be toxic to mice.[99] The analogs of the 6-thiopurines, MPR, MeMPR, TGR, and MeTGR were also all inactive.[98] The analogs of the ribonucleosides of 6-chloro, 6-methoxy-, 6-methylamino-, and 6-hydroxylamino purine were cytotoxic but were less active than C-Ado.[98] None of these compounds showed any activity against L1210 leukemia *in vivo*. The only analog of the cytotoxic 8-azapurine nucleosides that proved to be cytotoxic, carbocyclic 8-azaadenosine (C-8-azaAdo), is also the only carbocyclic purine analog that has shown *in vivo* antileukemic activity in mice.[100,101]

Table VI
Cytotoxicity and Antiviral Activity of Carbocyclic Purines

Carbocyclic Analog of	Cytotoxicity H.Ep. No. 2 Cells ID_{50} (μM)	Virus Rating[a]	
		HSV	Vaccinia
Adenosine	0.7	0.1	0.2
8-Azaadenosine	0.7	0.2	0.2
6-Thioinosine	>400	0.2	0
6-(Methylthio)purine ribonucleoside	90	2.9	2.9
O^6-Methylinosine	70[b]	2.2	1.9
N-Hydroxyadenosine	30	1.6	1.8
6-Chloropurine ribonucleoside	10	0.8	0.8
Inosine	>400	2.1	1.6
8-Azainosine	>400	1.5	1.2
N'-Methyladenosine	10	1.9	1.5

[a] See Ref. 102.
[b] KB Cells.

It is not too surprising that the more cytotoxic carbocyclics show little antiviral activity (Table VI), whereas the highly active antivirals are either not toxic or show only moderate toxicity to H. Ep.-2 cells.[102] The three most active compounds, the analogs of the ribonucleosides of 6-(methylthio)purine, 6-methoxypurine, and hypoxanthine, are as active as ara-A against both Herpes Simplex and Vaccinia viruses.

Carbocyclic analogs of a number of pyrimidine nucleosides have also been investigated. Of the uracil[103] and thymine[104] derivatives only the carbocyclic thymidine (C-TdR) showed any biologic activity giving modest increases in lifespan of L1210 leukemic mice at high dose levels. The carbocyclic analog (C-FUdR) of 2'-deoxy-5-fluorouridine is enzymatically phosphorylated to the monophosphate, which inhibits the incorporation of 2'-deoxyuridine into the DNA of cancer cells, presumably by the inhibition of thymidylate synthetase, but it is less potent than FUdR and much less toxic to L1210 cells in culture. The analog of 3'-deoxy-5-fluorouridine and 5-fluorouridine are even less cytotoxic. C-FUdR was inactive in tests against P-388 leukemia in mice.[105]

Of the pyrimidine analogs, the derivatives of cytosine are by far the most promising.[106–108] Although the analogs of 2'- and 3'-deoxycytidine were inactive,[108] both the 1-β-D-arabinofuranosyl cytosine (ara-C)[107] and cytidine[106] analogs are active against leukemia L1210 *in vivo*. In addition, the cytidine analog (carbodine) is active against RNA viruses such as human influenza type A viruses in the range of antiviral potency of ribavirin, but less potent than amantadine hydrochloride in concomitant assays.[109] The fact that carbodine is metabolized to its triphosphate in mammalian cells makes interference with viral RNA-dependent RNA

polymerization a likely possibility for its principal mode of action. The carbocyclic analogs of uridine—the deamination product of carbodine, 2'-deoxycytidine, 3'-deoxycytidine, N-methylcytidine, N,N-dimethylcytidine, and some related compounds were all inactive against PR-8 influenza virus *in vitro*.[109] Although reproducibly active *in vitro*, carbodine was ineffective against lethal influenza virus infections in mice under the conditions employed.

VI. PURINES AND PYRIMIDINES CONTAINING CHEMICALLY REACTIVE FUNCTIONS

An early attempt to combine an alkylating function with the purine nucleus resulted in failure: treatment of 2,2'-(2-purine-6-ylimino) diethanol with thionyl chloride gave, instead of the desired N,N-bis(2-chloroethyl)adenine,[110] the tricyclic derivative 9-(2-chloroethyl)-7,8-dihydro-9H-imidazo [2.1-*i*] purine hydrochloride,[111] which was biologically inert (Fig. 11). More success was encountered in attempts to

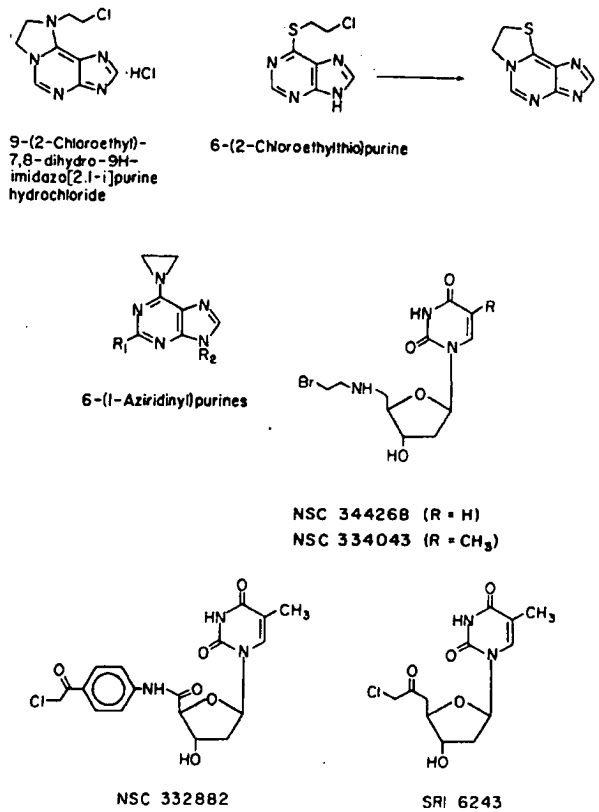


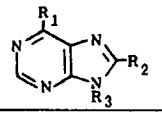
Figure 11. Purines and pyrimidines containing chemically reactive functions.

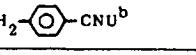
prepare the sulfur mustard analogs. Alkylation of MP with 1-bromo-2-chloroethane gave an active anticancer agent 6-(2-chloroethylthio) purine, which although easily cyclized to the inactive 7,8-dihydrothiazolo [2,3-*i*] purine, could be isolated in pure form.[112,113] 2-(2-Chloroethylthio)purine, similarly prepared from 2-mercaptopurine, was inactive, but 8-mercaptopurine gave only a mixture of the two possible tricyclic purines.[113]

The activity of the monofunctional alkylating agent N-(2,4-dinitrophenyl)aziridine provided the basis for the synthesis of some 6-(1-aziridinyl)purines, which showed activity against adenocarcinoma 755, a tumor not normally sensitive to alkylating agents.[114]

The activity of N-methyl-N-nitrosourea suggested the incorporation of this moiety into a purine structure: N-methyl-N-nitroso-N'-[2-(purin-6-ylthio)ethyl] urea (NSC 409293) was more active against leukemia L1210 *in vivo* than simple 6-(alkylthio)purines and substitution of a butyl group at N-9 destroyed activity (NSC 76790) (Table VII).[115] The importance of the atom attached to C-6 of the purine ring is indicated by the modest activity of the adenine derivative (NSC 69709). Purines substituted with a nitrosoureido side chain at C-8 or N-9 are only slightly active except for the derivative of N-(2-chloroethyl)-N-nitroso-N'-(4-tolyl)urea (NSC 125089), which was less active than other N-(2-

Table VII
Activity of Some Nitrosoureidopurines Against Leukemia



NSC No.	R_1	R_2	R_3	L1210 (ip 10^5 cells); R_x: ip, single dose: Optimal dose, mg/kg	% ILS[a]
409293	$MNU(CH_2)_2S$[b]	H	H	150	121
69709	$MNU(CH_2)_2NH$	H	H	400	26
76790	$MNU(CH_2)_2S$	H	Bu	300	9
105764	H	$MNU(CH_2)_2S$	H	375	28
77590	SH	H	$(CH_2)_2MNU$	150 200T	21
77591	SMe	H	$(CH_2)_2MNU$	Inactive	
75939	OH	H	$(CH_2)_2MNU$	100[c]	28
125089	OH	H		45	96

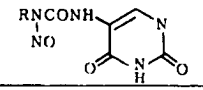
[a] Percent increase in lifespan.
[b] MNU = $NHCON(NO)CH_3$, CNU = $NHCON(NO)(CH_2)_2Cl$.
[c] Chronic.

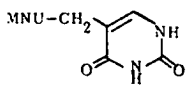
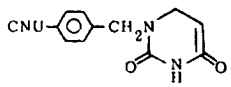
chloroethyl)-N-nitroso-N'-phenylureas with simple substituents at the 4-position of the phenyl ring.

Results with nitrosoureidopyrimidines were similar, the most active compound being the 5-[N-(2-chloroethyl)-N-nitrosoureido] uracil (NSC 80346) (Table VII). The corresponding methylnitrosourea (NSC 406024) was significantly more active than the thymine derivative (NSC 73314).[115] Thus, there are indications that the nature of the purine or pyrimidine and point of attachment are important to activity, although it is not clear that any of these compounds are functioning as antimetabolites or as carrier molecules for the active nitrosoureas. In fact, neither the uracil nor the MP moieties appear to enhance the activity of N-methyl-N-nitrosourea or N-(2-chloroethyl)-N-nitrosourea.

A number of nitrosoureidonucleosides,[116,117] prepared as active site directed irreversible enzyme inhibitors, proved to be unexpectedly chemically stable, which might explain their lack of biological activity. Certain phenyl carbamates, fluorosulfonylbenzamides, and diazoketones were also inactive.[118,119] More promising are the 5'-bromoacetamido-5'-deoxy derivatives of both uridine (NSC 344268) and thymidine (NSC 334043),[119] and the chloroketones NSC 332882 and SRI 6243,[120] which

Table VIII
Activity of Some Nitrosoureidouracils Against Leukemia L1210



NSC No.	R	L1210 (ip, 10^5 cells); R_x: ip, single dose: Optimal dose, mg/kg	% ILS[a]
80346	$Cl(CH_2)_2$	40	188
76282	$HO(CH_2)_2$	400	90
406024	Me	480	88
106916	$F(CH_2)_2$	20	78
89205	$MeO(CH_2)_2$	187	48
76248	$-(CH_2)_3-$	400	46[b]
73314		75	33
110803		90	107

[a] Percent increase in lifespan.
[b] R_x: ip, chronic.

have shown cytotoxicity and, in the case of the 5'-haloacetamides, *in vivo* activity against L1210.[121]

VII. DEAZAPURINES

Our studies on the effect of substituting a CH group for the nitrogens of the purine ring began with the preparation of the deaza analogs of MP.[122,123] The lack of activity of these derivatives indicated that none of them is converted to the ribonucleotide by a cellular phosphoribosyltransferase and suggested the preparation of deaza analogs of 6-(methylthio)purine ribonucleoside, which is activated by adenosine kinase. Unfortunately, neither 1- nor 3-deaza-6-(methylthio)purine ribonucleoside was shown to have significant cytotoxicity to H. Ep.-2 cells in culture or to be phosphorylated in them.[124,125] 1-Deaza-6-thioguanosine and 1-deaza-S-methyl-6-thioguanosine and the corresponding deazapurines were also inactive.[126,127] The synthesis[128-131] of a variety of other 1- and 3-deazapurines and 8-azapurines, however, yielded two interesting structures (Fig. 12). A derivative of 2,6-diamino-1-deazapurine, ethyl 7-[(diphenylmethyl)amino]-3H-imidazo [4,5-*b*] pyridine-5-carbamate (NSC 107390), gave a 60% increase in lifespan of L1210 leukemic mice, and results with related compounds indicated that both the ethoxycarbonyl and diphenylmethyl groups of this compound are necessary for activity, but little is known about its mechanism of action.[130]

2-Amino-6-chloro-1-deazapurine (ACD, Fig 12) is even more active, being highly cytotoxic to both H. Ep.-2 and L1210 cells in culture and giving an 80% increase in lifespan of leukemic mice.[70,129] This compound is converted to its ribonucleotide in L1210 cells, presumably by HPRT, since its toxicity is reversed by hypoxanthine. It has been shown to

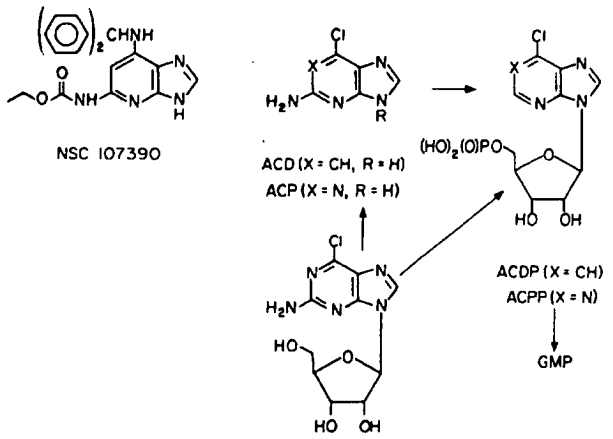


Figure 12. The 1-deazapurines.

inhibit inosinic dehydrogenase, but de novo purine nucleotide biosynthesis is more sensitive to it as indicated by reversal of its toxicity by 5-aminoimidazole-4-carboxamide only at the lower concentrations of drug.[132] Its mechanism of action is, then, similar to that of 2-amino-6-chloropurine (ACP),[133] but it is more toxic, probably because 2-amino-6-chloropurine ribonucleotide is converted to GMP by adenylate deaminase, and the deazapurine nucleotide is not. In addition, the ribonucleoside of 2-amino-6-chloropurine shows activity comparable to the base whereas the ribonucleoside of the deazapurine is inactive. Clearly ACD ribonucleoside[126] is neither phosphorylated nor cleaved back to the base. The activity of 2-amino-6-chloropurine ribonucleoside, on the other hand, could be due either to cleavage to the base or to phosphorylation.

Interest in the effects of the inhibition of adenosylhomocysteinase (SAHase), the enzyme that converts adenosylhomocysteine (SAHcy) to adenosine in cells (Fig. 13), caused Cantoni and Chiang to examine a number of our nucleosides for their ability to inhibit this enzyme.[134] Although several of these compounds did inhibit it, the most potent nucleoside found until recently (Table IX) was 3-deazaadenosine (3-deazaAR, Fig. 13),[135] for which we developed a practical synthesis.[136,137] 3-DeazaAR might be most aptly described as an alternative substrate since it not only competes with adenosine but is itself converted to 3-deazaadenosylhomocysteine, which along with SAHcy, inhibits competitively most of the methyltransferases that utilize adenosylmethionine (SAM) as the methyl donor (Fig. 13).[134] Perturbation of these biochemical transmethylations[96] may be responsible for the *in vitro* antimalarial, antiviral, and other effects of 3-deazaAR, which is not

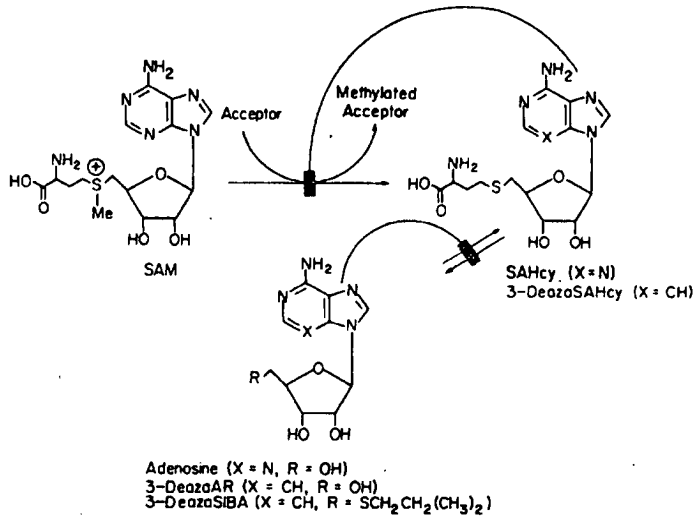


Figure 13. The locus of action of 3-deazaadenosine.

Table IX
Inhibition and Substrate Activity of Adenosine Analogs for AdoHcy Hydrolase

Compound	Inactivation K_i (uM)	Competitive Inhibition K_i (uM)	Substrate
C-Ado	--[a]	0.005	-
Ara-A	4.5	18	-
2-Cl-3-deaza-Ado	36	N.C.[b]	
3-Deaza-ara-A	54	86	
2-Cl-Ado	87	N.C.	-
2-F-ara-A	110	N.C.	
dAdo	174	N.C.	-
Nucleocidin	209	34	
2-Cl-ara-A	366	N.C.	
Purine ribonucleoside	--[c]	1390	+
7-Deaza-C-Ado	--[c]	N.D.[d]	
3-Deaza-C-Ado	N.I.[e]	3.0	+
3-Deaza-Ado	N.I.	4.0	+
8-Amino-Ado	N.I.	15	+
8-Aza-Ado	N.I.	190	+
N-Me-Ado	N.I.	190	+
Formycin	N.I.	280	+
2-Aza-3-deazaAdo	N.I.	340	+
Ado-5'-$CONH_2$	C[f]		-
SIBA	C		-
3-Deaza-SIBA	C		-
Tubercidin	N.I.	N.D.	-[g]

[a] Potent, K_I not accurately determined.
[b] N.C. = not competitive.
[c] Inactivates, K_I not accurately determined.
[d] N.D. = not determined.
[e] N.I. = no inactivation.
[f] Curvilinear inactivation.
[g] AdoHcy analog formed via SAM.

deaminated nor appreciably phosphorylated in cells. A derivative of 3-deazaAR,5'-deoxy-5'-(isobutylthio)-3-deazaadenosine (3-deazaSIBA), a weak noncompetitive inhibitor but not a substrate of SAHase, has selective antiviral activity against the Rous sarcoma virus in chick embryo cells, and against the Gross murine leukemia virus in mouse embryo cells, but the mechanism of the antiviral activity of this analog remains obscure.[138]

The finding that carbocyclic adenosine (C-Ado)(see above), which is rapidly phosphorylated and also deaminated in whole cells,[93] is the most potent inhibitor of SAHase (Table IX) thus far studied[94,95] led to the synthesis of the carbocyclic analog of 3-deazaadenosine (3-deaza-C-Ado, Fig. 14).[139] Studies with this compound showed that it is not deaminated

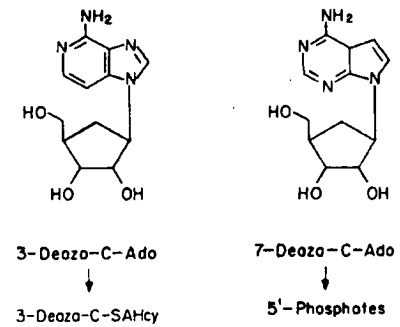


Figure 14. Carbocyclic analogs of deazapurine nucleosides.

or phosphorylated, but is as potent as 3-deazaadenosine in the inhibition of adenosylhomocysteinase and, in some cells, serves as an alternative substrate for the enzyme. It is more potent than 3-deazaadenosine against Herpes Simplex virus type 1, but inferior to ara-A. Against vaccinia, however, it is quite effective and clearly better than ara-A. It is the most potent inhibitor of vesicular stomatitis virus thus far studied.[140]

The impressive activity of 3-deaza-C-Ado prompted the synthesis of another deaza compound, the carbocyclic analog (7-deaza-C-Ado, Fig. 14) of tubercidin (7-deazaadenosine) by a published route.[141] 7-Deaza-C-Ado is highly cytotoxic to cells with adenosine kinase (AK^+) but not to cells deficient in this enzyme (AK^-), indicating that it must be phosphorylated to exert its effects, whereas 3-deaza-C-Ado does not, since it is only about fourfold more toxic to AK^+ than AK^- cells.

VIII. HALOPURINES

One of the smallest steric changes that can be made in the structure of a metabolite is the replacement of hydrogen by fluorine, since its van der Waals radius (1.35 Å) is very close to that of hydrogen (1.2 Å), while at the same time fluorine, the most electronegative atom found in organic compounds, should have a pronounced effect on the electronic configuration of the molecule. For this reason we elected to prepare 2-fluoroadenosine[142] and a series of related 2-fluoropurines (Fig. 15)[143]; the announcement of this synthesis was simultaneous with that of 5-fluorouracil.[144] Both 2-fluoroadenosine and its base, 2-fluoroadenine, are highly toxic to cells in culture (Table X), and the maximum tolerated chronic dose of 2-fluoroadenosine to C57 black mice implanted with adenocarcinoma 755 is only 1 mg/kg. As predicted, the powerful inductive effect of the fluorine at C-2 reduces the basic strength of adenine (pK_a 3.6) to such an extent that protonation of 2-fluoroadenine cannot be detected in 50% aqueous alcohol, whereas the acidic ionization is essentially unchanged. The biologic effect of this change in electron

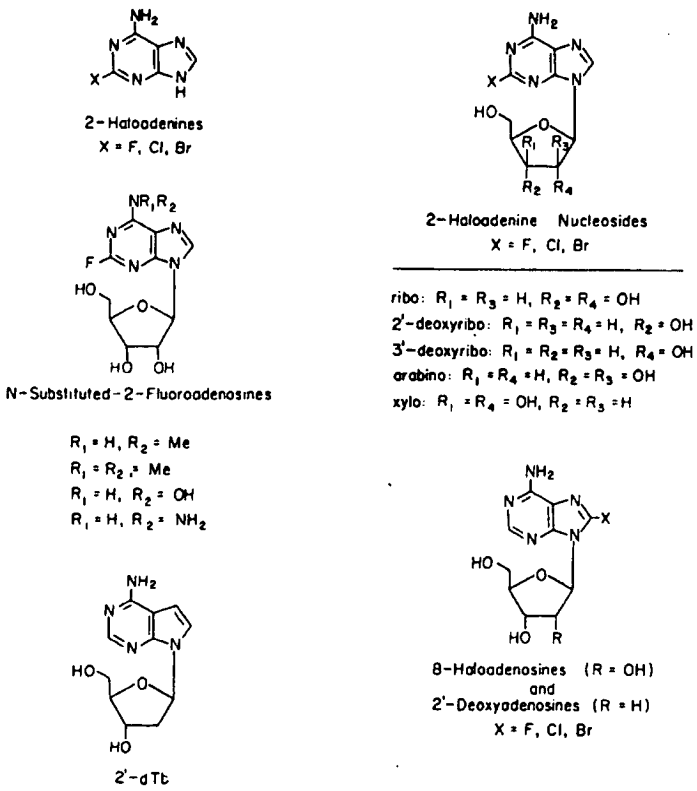


Figure 15. The halopurines and their nucleosides.

distribution is resistance of 2-fluoroadenosine to the action of adenosine deaminase[145,146] and enhancement of its phosphorylation by adenosine kinase.[147] In whole cells 2-fluoroadenylic acid is further phosphorylated to the di- and triphosphates,[148] but not incorporated into nucleic acids.[149] Neither 2-fluoroadenine nor 2-fluoroadenosine appear to have any selective cytotoxicity for neoplastic cells, nor is their mechanism of action as yet understood. 2-Fluoroadenine is converted to its ribonucleotide by adenine phosphoribosyltransferase (APRT) and cell lines lacking this enzyme are resistant to it.[150] Cells lacking adenosine kinase (AK) but not APRT are only 40-fold resistant to 2-fluoroadenosine, whereas the mutant lacking both enzymes is more than 2000-fold resistant, indicating that some cleavage of 2-fluoroadenosine must occur in mammalian cells even though adenosine phosphorylase activity is quite low in them.[151,152] Substitution of a methyl group on the amino group of 2-fluoroadenosine, which in the case of adenosine prevents its conversion to the diphosphate,[153] decreases its cytotoxicity by 1200-fold (Table XI), suggesting that its conversion to 2-fluoro-ATP is important to its biologic activity.[154]

The triacetate of 2-fluoroadenosine[154,155] is only one-seventh as toxic as 2-fluoroadenosine itself, indicating some resistance of this derivative to esterase cleavage of the O-acetyl groups. There is little difference in the cytotoxicity of N-methyladenosine, a poor substrate for ADA, and 2-fluoro-N-methyladenosine (Fig. 15) as might be expected, while the activity of N-hydroxyadenosine, a good substrate for ADA, is greatly increased by the 2-fluoro group. The instability of 2-fluoro-N-hydroxyadenosine made a direct comparison impossible, but based on the relative toxicities of 2-fluoroadenosine and its triacetate, the ratio of toxicity of N-hydroxyadenosine and its 2-fluoro derivative can be estimated to be at least 200. Substitution of 6-(methylthio)purine ribonucleoside by fluorine at C-2 decreases its toxicity about 70-fold as a result of its loss of activity as a substrate for AK (Table XI).

Substitution of fluorine at C-2 of 8-azaadenosine has the same effect that it has on adenosine in that, even though deamination proceeds at a measurable rate, it is greatly decreased. 8-Aza-2-fluoroadenosine, which is about one-half as cytotoxic as 8-azaadenosine, cannot be converted to 8-azaGR phosphates because of the fluorine at C-2, but could be incorporated into DNA as such.[156]

2-Chloroadenosine is only 1/350 and 2-bromoadenosine[157] only 1/6000 as cytotoxic as 2-fluoroadenosine even though these compounds are much more resistant to deamination (Table X).[146] This loss of activity results from the fact that they are poor substrates for AK.[147] Although the loss of substrate activity for the kinase may be a steric effect, this is not the case for ADA, since the affinities of the 2-haloadenosines for this enzyme, while lower than that of adenosine itself, increase not only with decreasing electronegativity of the halogen, but also the increasing size of the halogen.[146]

Two analogs of 2'-deoxyadenosine that are resistant to deamination, 2'-deoxy-2-fluoroadenosine (2-FAdR)[158] and 9-β-D-arabinofuranosyl-2-fluoroadenine (2-F-araA)[158,159] are cytotoxic to cells in culture,[160,161] and on the proper schedule, curative of L1210 leukemia in mice.[162,163] 2-FAdR is toxic to cells lacking APRT, to cells lacking adenosine kinase, and to cells lacking both enzymes,[158] but not to cells lacking 2'-deoxycytidine kinase (dCK).[160] 2-F-araA is also toxic to cells lacking AK,[158] but not to cells lacking dCK.[164] These compounds are substrates for dCK and not AK,[164] whereas ara-A is a substrate for both.[165] Consequently, a combination of ara-A and the ADA inhibitor 2'-deoxycoformycin is active against L1210/ara-C *in vivo* as it is against L1210/0, but 2-F-araA is not.[164] 2-F-araA is active, however, against P388 leukemia resistant to araA.[160] The mechanism of resistance of L1210 to ara-C is the loss of dCK; the mechanism of resistance of P388 to ara-A is not known but obviously does not involve loss of dCK. Both of these fluoro analogs are converted to their triphosphates in cells, and 2-F-araA is a good inhibitor of both nucleoside diphosphate reductase and DNA polymerase interfering effectively with DNA synthesis in treated cells.[167] The mechanism

Table X
Cytotoxicity of Nucleosides of the Haloadenines[a]

Compound	ID_{50}, uM			Degree of Resistance				
	H.Ep. No. 2	CCRF-CEM[b]	WI-L2[c]	APRT$^-$[d]	AK$^-$[d]	APRT$^-$/AK$^-$[d]	dCK$^-$[e]	AK$^-$/dCK$^-$[e]
Adenine	100							
2-Fluoroadenine	0.03			>2000	1	>2000		
2-Chloroadenine	10							
2-Bromoadenine								
Adenosine	1							
2-Fluoroadenosine	0.02			20	1-2	>2000		
2-Chloroadenosine	7			1	>10	> 10		
2-Bromoadenosine	120			(1)				
2'-Deoxyadenosine	10[f]	3[f,g]	50[f]					
2'-Deoxy-2-fluoroadenosine	0.2	0.2[g,h]	0.1-0.4	1-2	1	1-2	70	100
2-Chloro-2'-deoxyadenosine		0.003, 0.02[h]	0.04		1		>60	>60
2-Bromo-2'-deoxyadenosine		0.02[h]						
9-β-D-Arabinofuranosyladenine	>300	0.4[f]	5[f]					
9-β-D-Arabinofuranosyl-2-fluoroadenine	8	0.5	3					
9-β-D-Arabinofuranosyl-2-chloroadenine		10	70					
9-β-D-Arabinofuranosyl-2-bromoadenine								
9-α-D-Arabinofuranosyladenine	40				>80			
9-α-D-Arabinofuranosyl-2-fluoroadenine	40							
9-α-D-Arabinofuranosyl-2-chloroadenine								
3'-Deoxyadenosine	80			1	1			
3'-Deoxy-2-fluoroadenosine	2			20	1-2	20		
3'-Deoxy-2-chloroadenosine								
3'-Acetamido 3'-deoxyadenosine								
3'-Acetamido-3'-deoxy-2-fluoroadenosine	20			1		> 3		
3'-Acetamido-3'-deoxy-2-chloroadenosine	> 60							

9-β-D-Xylofuranosyladenine	30							
2-Fluoro-9-β-D-xylofuranosyladenine	40			> 100				
5'-Deoxy-2-fluoroadenosine	0.1					> 400		
5'-Deoxy-5'-ethylthio-2 fluoroadenosine	0.2				10[i]			
8-Fluoroadenosine	0.6							
8-Chloroadenosine								
8-Bromoadenosine	9[j]							
2'-Deoxy-8-Fluoroadenosine								
8-Chloro-2'-deoxyadenosine	>100							
8-Bromo-2'-deoxyadenosine	370[j]	>100	>100					
2'-Deoxy-7-deazaadenosine		>100	33					

[a] For definitions of terms and resistant cell lines see Table IV.
[b] Human T lymphoblastoid cells.
[c] Human B lymphoblastoid cells.
[d] H. Ep. No. 2 sublines. See Table II.
[e] WI-L2 sublines (dCK$^-$ = 2'-deoxycytidine kinase deficient).
[f] Plus 2'-deoxycoformycin.
[g] Unless otherwise stated data is from Ref. 160.
[h] Ref. 161.
[i] Plus 2'-deoxycoformycin >60.
[j] KB cells.

Table XI
Cytotoxicity of N-Substituted Derivatives of 2-Fluoradenosine to H. Ep-2 Cells

Compound	ID_{50} (μM)
2-Fluoroadenosine	0.020
2-Fluoroadenosine triacetate	0.15
N-Methyladenosine	14
N,N-Dimethyladenosine	>340
2-Fluoro-N-methyladenosine (11)	23
2-Fluoro-N,N-dimethyladenosine (12)	>130
N-Hydroxyadenosine	32
2-Fluoro-N-hydroxyadenosine triacetate (9)	1
N-Aminoadenosine	1.8
N-Amino-2-fluoroadenosine triacetate (10)	6.7
2-Fluoro-6-(methylthio)purine ribonucleoside (5)	19
6-(Methylthio)purine ribonucleoside	0.30
2,6-Difluoropurine ribonucleoside triacetate (6)	31

of action of 2-FAdR is likely to be similar. 2-Bromo- and 2-chloro-2′-deoxyadenosine are even more resistant to adenosine deaminase than the fluoro compound, as are the ribonucleosides; but, in contrast to the ribonucleosides, are more cytotoxic.[160,161] They are also highly active against leukemia L1210 *in vivo*.[160,163] It seems logical that the steric effects of halogen substitution at C-2 of the purine would greatly affect the binding of these compounds to AK, but not to dCK, the normal substrate for which is a pyrimidine and not a purine, but it is harder to understand the enhanced substrate efficiencies[168] (which has been demonstrated only in the case of fluorine but which may be inferred from the greater cytotoxicity of the 2′-chloro and 2′-bromo compounds) which must result from electronic effects. Thus, the difference in activity of the 2′-deoxy-2-haloadenosines and the activity (or lack of it) of the 2-haloadenosines is explainable by the difference in substrate requirements of the activating enzymes, AK and dCK.

The cytotoxicity of 3-deoxyadenosine is enhanced by 2′-deoxycoformycin, which is less effective with 9-β-D-xylofuranosyladenine, even though both compounds are deaminated by adenosine deaminase at a significant rate.[169] Similarly, 3′-deoxy-2-fluoroadenosine is 40 times as cytotoxic as 3′-deoxyadenosine, whereas insertion of fluorine into the 2-position of 9-β-D-xylofuranosyladenine does not alter its activity.[158] Cell lines lacking AK still respond to 3′-deoxyadenosine, which is a substrate for this enzyme, indicating that either it is cleaved to adenine, the toxicity of which is great enough to account for the rather high ID_{50} observed in either cell line, or is a substrate for another kinase also. The 20-fold resistance of cells lacking APRT, but not those lacking AK, to the fluoro analog, which is at best a very poor substrate for AK, indicates that it too is at least partially cleaved to 2-fluoroadenine. Neither 3′-acetamido-3′-deoxyadenosine nor its 2-chloro derivative are cytotoxic, but the 2-fluoro derivative has moderate activity and is apparently phosphorylated by AK since deficient cells are resistant to this analog.[170] Two 5′-deoxy derivatives of 2-fluoroadenosine, the 5′-deoxy-[171] and 5′-deoxy-5′-ethylthio-[172] ribonucleosides are both quite cytotoxic to H. Ep.-2 cells except sublines lacking APRT, clearly indicating efficient cleavage of these nucleosides occurs in sensitive cells. 5′-Deoxy-5′-ethylthio-2-fluoroadenosine is a good substrate for 5′-(methylthio)-5′-deoxyadenosine phosphorylase (MTAP) and cells containing this enzyme are sensitive to this compound[173] while those that do not, like L1210 leukemia, fail to respond.[172] Since 5′-deoxyadenosine is also a good substrate for this enzyme it is clear that the fluoro analog is activated by the same mechanism. Since these compounds are relatively nontoxic to mice, they may represent a useful prodrug type for the treatment of neoplasms having MTAP.

The lack of activity of the 2′-deoxytubercidin (2′-dTb),[160] a different kind of ADA-resistant analog, probably indicates that it is not phosphorylated in cells, underscoring the difference in substrate requirements of the different kinases. In contrast, 6-methylpurine ribonucleoside is quite cytotoxic and its metabolism is similar to that of the ADA-resistant tubercidin and 2-fluoroadenosine.[174]

8-Haloadenine nucleosides are also resistant to adenosine deaminase, but apparently less so than the 2-halo compounds. Data on this series is incomplete but neither 8-chloro- nor 8-bromo-2′-deoxyadenosine is cytotoxic, presumably because they are not substrates for dCK for steric reasons. 8-Fluoroadenosine is tenfold less cytotoxic to AK^- cells than to AK^+ cells. 2′-Deoxycoformycin has little effect on the cytotoxicity to AK^+ cells but eliminates its activity in the AK^- cells, indicating that 8-fluoroadenosine is phosphorylated by AK, but in its absence is deaminated to 8-fluoroinosine, which is about 1/10 as toxic as the adenosine. None of the other halohypoxanthine nucleosides that have been evaluated are cytotoxic.[175]

These studies were supported in the main by the National Cancer Institute, DHHS, the American Cancer Society, the C. F. Kettering Foundation, and the Alfred P. Sloan Foundation.

REFERENCES

1. D. D. Woods, *Br. J. Exp. Path.*, **21**, 74 (1940).
2. P. Fildes, *Lancet*, **1**, 955 (1940).
3. G. H. Hitchings, E. A. Falco, and M. B. Sherwood, *Science*, **102**, 251 (1945).
4. R. O. Roblin, *Chem. Rev.*, **38**, 255 (1946).
5. G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).
6. D. A. Clarke, F. S. Philips, S. S. Steinberg, C. C. Stock, G. B. Elion, and G. H. Hitchings, *Cancer Res.*, **13**, 593 (1953).
7. H. E. Skipper, J. R. Thomson, G. B. Elion, and G. H. Hitchings, *Cancer Res.*, **14**, 294 (1954).

8. J. H. Burchenal, M. L. Murphy, R. R. Ellison, M. P. Sykes, T. C. Tan, L. A. Leone, D. A. Karnofsky, L. F. Crarer, H. W. Dargeon, and C. P. Rhoads, *Blood.* **8**, 965 (1953).
9. R. B. Livingston and S. K. Carter, *Single Agents in Cancer Chemotherapy*, IFI/Plenum, New York, 1970, p. 173.
10. R. W. Brockman, M. C. Sparks, and M. S. Simpson, *Biochim. Biophys. Acta.* **26**, 671 (1957).
11. R. W. Brockman, C. Sparks, M. S. Simpson, and H. E. Skipper, *Biochem. Pharmacol.*, **2**, 77 (1959).
12. R. W. Brockman, C. Sparks, D. J. Hitchison, and H. E. Skipper, *Cancer Res.*, **19**, 177 (1959).
13. R. W. Brockman, *Cancer Res.*, **20**, 643 (1960).
14. R. W. Brockman, *Antineoplastic and Immunosuppressive Agents*, Handbook of Experimental Pharmacology, A. C. Sartorelli and D. G. Johns, Eds., Springer-Verlag, Berlin, 1974, p. 352.
15. J. F. Henderson and R. W. Brockman, 27th Annual Symposium on Fundamental Cancer Research, *Pharmacological Basis of Cancer Chemotherapy*, Williams and Wilkins, Baltimore, 1975, p. 629.
16. J. A. Johnson, Jr. and H. J. Thomas, *J. Am. Chem. Soc.*, **78**, 3863 (1956).
17. J. A. Johnson, Jr., H. J. Thomas, and H. J. Schaeffer, *J. Am. Chem. Soc.*, **80**, 699 (1958).
18. H. E. Skipper, J. R. Thomson, D. J. Hutchison, F. M. Schabel, Jr., and J. J. Johnson, Jr., *Proc. Soc. Exp. Biol. Med.*, **95**, 135 (1957).
19. H. E. Skipper, J. A. Montgomery, J. R. Thomson, and F. M. Schabel, Jr., *Cancer Res.*, **19**, 425 (1959).
20. F. M. Schabel, Jr., J. A. Montgomery, H. E. Skipper, W. R. Laster, Jr., and J. R. Thomson, *Cancer Res.*, **21**, 690 (1961).
21. A. Hampton, J. J. Biesele, A. E. Moore, and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 5695 (1956).
22. J. A. Montgomery, T. P. Johnston, A. Gallagher, C. R. Stringfellow, and F. M. Schabel, Jr., *J. Med. Pharm. Chem.*, **3**, 265 (1961).
23. L. L. Bennett, Jr., R. W. Brockman, H. P. Schnebli, S. Chumley, G. J. Dixon, F. M. Schabel, Jr., E. A. Dulmadge, H. E. Skipper, J. A. Montgomery, and H. J. Thomas, *Nature*, **205**, 1276 (1965).
24. F. M. Schabel, Jr., W. R. Laster, Jr., and H. E. Skipper, *Cancer Chemother. Rep.*, **51**, 11 (1967).
25. G. P. Bodey, H. S. Brodovsky, A. A. Isassi, M. L. Samuels, and E. J. Freireich, *Cancer Chemother. Rep.*, **52**, 315 (1968).
26. J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **26**, 1926 (1961).
27. J. A. Montgomery, F. M. Schabel, Jr., and H. E. Skipper, *Cancer Res.*, **22**, 504 (1962).
28. L. L. Bennett, Jr., and J. A. Montgomery, *Methods in Cancer Res.*, H. Busch, Ed., Academic, New York and London, 1967, Vol. 3, Chapter IX, p. 549.
29. J. A. Montgomery and R. F. Struck, *Progress in Drug Research*, E. Jucker, Ed., Birkhäuser Verlag, Basel and Stuttgart, 1973, Vol. 17, p. 322.
30. D. L. Hill and L. L. Bennett, Jr., *Biochemistry*, **8**, 122 (1969).
31. L. L. Bennett, Jr. and D. J. Adamson, *Biochem. Pharmacol.*, **19**, 2172 (1970).
32. L. L. Bennett, Jr. and P. W. Allan, *Cancer Res.*, **31**, 152 (1971).
33. L. L. Bennett, Jr., L. Simpson, J. Golden, and T. L. Barker, *Cancer Res.*, **23**, 1574 (1963).
34. H. T. Baker and L. L. Bennett, Jr., *Biochim. Biophys. Acta.* **80**, 497 (1964).
35. G. B. Elion, *Fed. Proc.*, **26**, 898 (1967).
36. D. M. Tidd and A. R. P. Paterson, *Cancer Res.*, **34**, 738 (1974).
37. J. A. Nelson, J. W. Carpenter, L. M. Rose, and D. J. Adamson, *Cancer Res.*, **35**, 2872 (1975).
38. L. L. Bennett, Jr. and P. W. Allan, *Cancer Res.*, **31**, 152 (1971).
39. J. A. Montgomery, T. P. Johnston, and Y. F. Shealy, *Burger's Medicinal Chemistry*, M. E. Wolff, Ed., Wiley, New York, 1979, 4th Edition, Part II, Chap. 24, p. 595.

40. J. A. Montgomery, H. J. Thomas, and H. J. Schaeffer, *J. Org. Chem.*, **26**, 1929 (1961).
41. H. J. Thomas and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 24 (1962).
42. H. J. Thomas and J. A. Montgomery, *J. Med. Chem.*, **11**, 44 (1968).
43. J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **10**, 1163 (1967).
44. J. A. Montgomery, G. J. Dixon, E. A. Dulmadge, H. J. Thomas, R. W. Brockman, and H. E. Skipper, *Nature*, **199**, 769 (1963).
45. J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **79**, 5238 (1957).
46. J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **80**, 409 (1958).
47. C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 866 (1962).
48. G. G. Kelley, G. P. Wheeler, and J. A. Montgomery, *Cancer Res.*, **22**, 329 (1962).
49. C. B. Johnson, W. B. Frommeyer, Jr., W. J. Hammack, and C. E. Butterworth, Jr., *Cancer Chemother. Rep.*, **20**, 137 (1962).
50. J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **26**, 4469 (1961).
51. J. A. Montgomery, H. J. Thomas, and K. Hewson, *J. Med. Chem.*, **15**, 1189 (1972).
52. J. A. Montgomery and A. G. Laseter, unpublished data.
53. C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Chem.*, **11**, 41 (1968).
54. C. Temple, Jr., B. H. Smith, and J. A. Montgomery, *J. Org. Chem.*, **33**, 530 (1968).
55. C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Chem.*, **15**, 441 (1972).
56. C. Temple, Jr., A. G. Laseter, and J. A. Montgomery, *J. Med. Chem.*, **11**, 1213 (1968).
57. C. Temple, Jr., A. G. Laseter, and J. A. Montgomery, *J. Heterocycl. Chem.*, **5**, 711 (1968).
58. G. W. Kidder, V. C. Dewey, R. E. Parks, Jr., and G. L. Woodside, *Science*, **109**, 511 (1949).
59. J. Davoll, *J. Chem. Soc.*, 1593 (1958).
60. J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **36**, 1962 (1971).
61. J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **15**, 305 (1972).
62. L. L. Bennett, Jr., M. H. Vail, P. W. Allan, and W. R. Laster, Jr., *Cancer Res.*, **33**, 465 (1973).
63. J. A. Montgomery and H. J. Thomas, *The Purines-Theory and Experiment*, The Jerusalem Symposia on Quantum Chemistry and Biochemistry, E. D. Bergmann and B. Pullman, Eds., The Israel Academy of Sciences and Humanities, 1972, Vol. IV, p. 446.
64. J. A. Montgomery, R. D. Elliott, and H. J. Thomas, *Ann. N.Y. Acad. Sci.*, **255**, 292 (1975).
65. L. L. Bennett, Jr., D. L. Hill, and P. W. Allan, *Biochem. Pharmacol.*, **27**, 83 (1978).
66. L. L. Bennett, Jr., and P. W. Allan, *Cancer Res.*, **36**, 3917 (1976).
67. J. A. Montgomery, H. J. Thomas, and S. J. Clayton, *J. Heterocycl. Chem.*, **7**, 215 (1970).
68. J. A. Montgomery and R. D. Elliott, *J. Chem. Soc. Chem. Commun.*, 1279 (1972).
69. R. D. Elliott and J. A. Montgomery, *J. Med. Chem.*, **20**, 116 (1977).
70. J. A. Montgomery, R. D. Elliott, P. W. Allan, L. M. Rose, and L. L. Bennett, Jr., *Advances in Enzyme Regulation*, G. Weber, Ed., Pergamon Press, New York, 1979, Vol. 17, p. 419.
71. L. L. Bennett, Jr., J. A. Montgomery, R. W. Brockman, and Y. F. Shealy, *Advances in Enzyme Regulation*, G. Weber, Ed., Pergamon, New York, 1978, Vol. 16, p. 255.
72. L. L. Bennett, Jr., L. M. Rose, P. W. Allan, D. Smithers, D. J. Adamson, R. D. Elliott, and J. A. Montgomery, *Mol. Pharmacol.*, **16**, 981 (1979).
73. R. D. Elliott and J. A. Montgomery, *J. Med. Chem.*, **19**, 1186 (1976).
74. L. L. Bennett, Jr., P. W. Allan, R. D. Elliott, and J. A. Montgomery, *Biochem. Pharmacol.*, **28**, 948 (1979).
75. J. A. Montgomery and H. J. Thomas, *Chem. Commun.*, 458 (1969).
76. J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **15**, 182 (1972).
77. J. A. Montgomery, A. G. Laseter, A. T. Shortnacy, S. J. Clayton, and H. J. Thomas, *J. Med. Chem.*, **18**, 564 (1975).
78. L. L. Bennett, Jr., P. W. Allan, J. W. Carpenter, and D. L. Hill, *Biochem. Pharmacol.*, **25**, 517 (1976).
79. W. M. Shannon, A. T. Shortnacy, G. Arnett, and J. A. Montgomery, *J. Med. Chem.*, **17**, 361 (1974).
80. F. M. Schabel, Jr., *Chemotherapy*, **13**, 321 (1968).
81. G. A. LePage, L. W. Worth, and A. P. Kimball, *Cancer Res.*, **36**, 1481 (1976).

82. F. M. Schabel, Jr., M. W. Trader, and W. R. Laster, Jr., *Proc. Am. Assoc. Cancer Res.*, **17**, 46 (1976).
83. J. A. Montgomery and H. J. Thomas, *J. Labelled Compd. Radiopharm.*, XV (Suppl.), 727 (1978).
84. L. L. Bennett, Jr., P. W. Allan, D. L. Hill, H. J. Thomas, and J. W. Carpenter, *Mol. Pharmacol.*, **12**, 242 (1976).
85. L. L. Bennett, Jr., P. W. Allan, S. C. Shaddix, W. M. Shannon, G. Arnett, L. Westbrook, J. C. Drach, and C. M. Reinke, *Biochem. Pharmacol.*, **30**, 2325 (1981).
86. J. A. Montgomery, A. T. Shortnacy, G. Arnett, and W. M. Shannon, *J. Med. Chem.*, **20**, 401 (1977); J. A. Montgomery and H. J. Thomas, *J. Heterocycl. Chem.*, **16**, 353 (1979).
87. L. L. Bennett, Jr. and D. L. Hill, *Mol. Pharmacol.*, **11**, 803 (1975).
88. R. L. Miller, D. L. Adamezyk, W. H. Miller, G. W. Koszalka, J. L. Rideout, L. M. Beacham, III, E. Y. Chao, J. J. Haggerty, T. A. Krenitsky, and G. B. Elion, *J. Biol. Chem.*, **254**, 2346 (1979).
89. Y. F. Shealy and J. D. Clayton, *J. Am. Chem. Soc.*, **88**, 3885 (1966).
90. Y. F. Shealy and J. D. Clayton, *J. Am. Chem. Soc.*, **88**, 3885 (1966).
91. Y. F. Shealy, M. C. Thorpe, W. C. Coburn, Jr., and J. D. Clayton, *Chem. Pharm. Bull.*, **28**, 3114 (1980).
92. D. L. Hill, S. Straight, P. W. Allan, and L. L. Bennett, Jr., *Mol. Pharmacol.*, **7**, 375 (1971).
93. L. L. Bennett, Jr., P. W. Allan, and D. L. Hill, *Mol. Pharmacol.*, **4**, 208 (1968).
94. A. Guranowski, J. A. Montgomery, G. L. Cantoni, and P. K. Chiang, *Biochemistry*, **20**, 110 (1981).
95. P. K. Chiang, A. Guranowski, and J. E. Segall, *Arch. Biochem. Biophys.*, **207**, 175 (1981).
96. P. K. Chiang and G. L. Cantoni, *Biochem. Pharmacol.*, **28**, 1897 (1979).
97. Y. F. Shealy and C. A. O'Dell, *Tetrahedron Lett.*, 2231 (1969).
98. Y. F. Shealy and J. D. Clayton, *J. Pharm. Sci.*, **62**, 1252 (1973).
99. Y. F. Shealy and J. D. Clayton, *J. Pharm. Sci.*, **62**, 1432 (1973).
100. Y. F. Shealy and J. D. Clayton, *J. Pharm Sci.*, **62**, 858 (1973).
101. Y. F. Shealy, J. D. Clayton, and C. A. O'Dell, *J. Heterocycl. Chem.*, **10**, 601 (1973).
102. L. L. Bennett, Jr., W. M. Shannon, P. W. Allan, and G. Arnett, *Annals of the New York Academy of Sciences*, 1975, Vol. 255, p. 342.
103. Y. F. Shealy and C. A. O'Dell, *J. Heterocycl. Chem.*, **13**, 1015 (1976).
104. Y. F. Shealy, C. A. O'Dell, and M. C. Thorpe, *Heterocycl. Chem.*, **18**, 383 (1981).
105. Y. F. Shealy, J. L. Frye, N. F. DuBois, S. C. Shaddix, and R. W. Brockman, *J. Med. Chem.*, **24**, 1083 (1981).
106. Y. F. Shealy and C. A. O'Dell, *J. Heterocycl. Chem.*, **13**, 1353 (1976).
107. Y. F. Shealy and C. A. O'Dell, *J. Pharm. Sci.*, **68**, 668 (1979).
108. Y. F. Shealy and C. A. O'Dell, *J. Heterocycl. Chem.*, **17**, 353 (1980).
109. W. M. Shannon, G. Arnett, L. Westbrook, Y. F. Shealy, C. A. O'Dell, and R. W. Brockman, *Antimicrob. Agnets Chemother.*, **20**, 769 (1981).
110. G. Huber, *Angew. Chem.*, **68**, 706 (1956).
111. T. P. Johnston, A. L. Fikes, and J. A. Montgomery, *J. Org. Chem.*, **27**, 973 (1962).
112. T. P. Johnston, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958).
113. R. W. Balsiger, A. L. Fikes, T. P. Johnston, and J. A. Montgomery, *J. Org. Chem.*, **26**, 3446 (1961).
114. J. A. Montgomery, K. Hewson, and C. Temple, Jr., *J. Med. Pharm. Chem.*, **5**, 15 (1962).
115. T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, *J. Med. Chem.*, **6**, 669 (1963).
116. J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **22**, 1109 (1979).
117. J. A. Montgomery, H. J. Thomas, R. W. Brockman, and G. P. Wheeler, *J. Med. Chem.*, **24**, 184 (1981).
118. J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **46**, 594 (1981).
119. R. D. Elliott, R. W. Brockman, and J. A. Montgomery, *J. Med. Chem.*, **24**, 350 (1981).
120. J. A. Montgomery and H. J. Thomas, *Nucleic Acid Research*, Symposium Series No. 9, 1981, p. 95.
121. R. D. Elliott and J. A. Montgomery, unpublished data.

122. J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **30**, 1528 (1965).
123. J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **8**, 708 (1965).
124. J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **9**, 105 (1966).
125. J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **9**, 354 (1966).
126. R. D. Elliott and J. A. Montgomery, *J. Med. Chem.*, **21**, 112 (1978).
127. C. Temple, Jr., B. H. Smith, C. L. Kussner, and J. A. Montgomery, *J. Org. Chem.*, **41**, 3874 (1976).
128. C. Temple, Jr., B. H. Smith, and J. A. Montgomery, *J. Org. Chem.*, **37**, 3601 (1972).
129. C. Temple, Jr., B. H. Smith, and J. A. Montgomery, *J. Org. Chem.*, **38**, 613 (1973).
130. C. Temple, Jr., B. H. Smith, R. D. Elliott, and J. A. Montgomery, *J. Med. Chem.*, **16**, 292 (1973).
131. C. Temple, Jr., B. H. Smith, and J. A. Montgomery, *J. Org. Chem.*, **38**, 1095 (1973).
132. L. L. Bennett, Jr., D. Smithers, N. F. DuBois, J. E. Hughes, and R. W. Brockman, *Proc. Am. Assoc. Cancer Res. and ASCO*, **22**, 27 (1981).
133. A. C. Sartorelli, J. H. Anderson, and B. A. Booth, *Biochem. Pharmacol.*, **17**, 37 (1968).
134. P. K. Chiang, H. H. Richards, and G. L. Cantoni, *Mol. Pharmacol.*, **13**, 939 (1977).
135. R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Biochemistry*, **5**, 756 (1966).
136. J. A. Montgomery, A. T. Shortnacy, and S. D. Clayton, *J. Heterocycl. Chem.*, **14**, 195 (1977).
137. J. A. Montgomery and H. J. Thomas, *J. Labelled Compd. Radiopharm.*, XV (Suppl.), 539 (1978).
138. P. K. Chiang, G. L. Cantoni, J. P. Bader, W. M. Shannon, H. J. Thomas, and J. A. Montgomery, *Biochem. Biophys. Res. Commun.*, **82**, 417 (1978).
139. J. A. Montgomery, S. J. Clayton, H. J. Thomas, W. M. Shannon, G. Arnett, A. J. Bodner, I.-K. Kim, G. L. Cantoni, and P. K. Chiang, *J. Med. Chem.*, in press.
140. E. DeClerq, unpublished data.
141. J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **10**, 665 (1967).
142. J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.*, **82**, 463 (1960).
143. J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.*, **79**, 4559 (1957).
144. R. Duschinsky, E. Pleven, and C. Heidelberger, *J. Am. Chem. Soc.*, **79**, 4559 (1957).
145. O. P. Chilson and J. R. Fisher, *Arch. Biochem. Biophys.*, **102**, 77 (1963).
146. M. H. Maguire and M. K. Sim, *Eur. J. Biochem.*, **23**, 22 (1971).
147. H. P. Schnebli, D. L. Hill, and L. L. Bennett, Jr., *J. Biol. Chem.*, **242**, 1997 (1967).
148. H. T. Shigeura, G. E. Boxer, S. D. Sampson, and M. L. Meloni, *Arch. Biochem. Biophys.*, **111**, 713 (1965).
149. L. L. Bennett, Jr., unpublished data.
150. L. L. Bennett, Jr., M. H. Vail, S. Chumley, and J. A. Montgomery, *Biochem. Pharmacol.*, **15**, 1719 (1966).
151. L. L. Bennett, Jr., H. P. Schnebli, M. H. Vail, P. W. Allan, and J. A. Montgomery, *Mol. Pharmacol.*, **2**, 432 (1966).
152. L. L. Bennett, Jr., M. H. Vail, P. W. Allan, and S. C. Shaddix, *Biochem. Pharmacol.*, **22**, 1221 (1973).
153. H. T. Shigeura, S. D. Sampson, and M. L. Meloni, *Arch. Biochem. Biophys.*, **115**, 462 (1966).
154. J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **13**, 427 (1970).
155. J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **33**, 432 (1968).
156. J. A. Secrist III, A. T. Shortnacy, and J. A. Montgomery, *Abstracts of the 183rd Meeting of the American Chemical Society*, Las Vegas, March 28–April 2, 1982.
157. J. A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, **1**, 213 (1964).
158. J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **12**, 498 (1969).
159. J. A. Montgomery, S. D. Clayton, and A. T. Shortnacy, *J. Heterocycl. Chem.*, **16**, 157 (1979).
160. D. A. Carson, D. B. Wasson, J. Kaye, B. Ullman, D. W. Martin, Jr., R. K. Robins, and J. A. Montgomery, *Proc. Natl. Acad. Sci.*, **77**, 6865 (1980).

161. M.-C. Huang, K. Hatfield, A. W. Roetker, J. A. Montgomery, and R. L. Blakley, *Biochem. Pharmacol.*, 30, 2663 (1981).
162. R. W. Brockman, F. M. Schabel, Jr., and J. A. Montgomery, *Biochem. Pharmacol.*, 26, 2193 (1977).
163. J. A. Montgomery and M. W. Trader, unpublished data.
164. R. W. Brockman, Y.-C. Cheng, F. M. Schabel, Jr., and J. A. Montgomery, *Cancer Res.*, 40, 3610 (1980).
165. V. L. Verhoef, J. Sarup, and A. Fridland, *Cancer Res.*, 41, 4478 (1981).
166. F. M. Schabel, Jr., unpublished data.
167. L. White, S. C. Shaddix, Y.-C. Cheng, R. W. Brockman, and L. L. Bennett, Jr., *Proc. Am. Assoc. Cancer Res.*, 22, 33 (1981).
168. T. A. Krenitsky, J. V. Tuttle, G. W. Koszalka, I. S. Chen, L. M. Beacham, III, J. L. Rideout, and G. B. Elion, *J. Biol. Chem.*, 251, 4055 (1976).
169. R. H. Adamson, D. W. Zaharevitz, and D. G. Johns, *Pharmacology*, 15, 84 (1977).
170. J. A. Montgomery, K. Hewson, and A. G. Laseter, *J. Med. Chem.*, 18, 571 (1975).
171. J. A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, 9, 445 (1972).
172. J. A. Montgomery, A. T. Shortnacy, and H. J. Thomas, *J. Med. Chem.*, 17, 1197 (1974).
173. T. M. Savarese, D. L. Dexter, E. N. Spremulli, G. W. Crabtree, S. H. Chu, J. A. Montgomery, R. E. Parks, Jr., and P. Calabresi, *Proc. Am. Assoc. Cancer Res. and ASCO.* 22, 209 (1981).
174. J. A. Montgomery and K. Hewson, *J. Med. Chem.*, 11, 48 (1968).
175. J. A. Secrist III, J. A. Montgomery, B. J. Bowdon, and L. L. Bennett, Jr., *Abstracts of the 183rd Meeting of the American Chemical Society.* Las Vegas, March 28–April 2, 1982.

Role of Prostaglandins in the Regulation of Gastrointestinal Functions

Camille F. Nassar

Department of Physiology, Faculty of Medicine, American University of Beirut, Beirut, Lebanon

I. INTRODUCTION

Prostaglandins (PGs), a group of cyclic hydroxy fatty acids, are highly active in a variety of biological systems, and have been investigated extensively.[1-4] The story of the discovery of prostaglandins begins in 1930 when R. Kurzok and C. Lieb, New York gynecologists, found that after artificial insemination some women underwent violent uterine contractions.[5] Sometime later, Goldblatt in England[6] and von Euler in Sweden[7] independently described some of the actions of seminal plasma on smooth muscle; and von Euler, obtaining his material from seminal fluid and from lipid extracts of sheep vesicular glands named it "prostaglandin.[7] In 1960, Bergström and Sjovall[8] were able to crystallize two prostaglandins. Since that time at least some 16 "natural" prostaglandin compounds have been isolated and described. All of these compounds are 20-carbon chain, unsaturated, cyclic fatty acids in which minor molecular changes can result in a compound with actions directly opposite to those of a structurally similar compound.

Recent reviews of the actions of prostaglandins have dealt extensively or entirely with the effect of prostaglandins on the metabolic function of the endocrine or reproductive glands, or on the central nervous system, the cardiovascular and renal functions. However, a full detailed review of the action of prostaglandins on the gastrointestinal tract has not been published. Therefore, a full detailed survey on the role of PGs in regulating the gastrointestinal functions is justified at the present time.

Medical Research Reviews, Vol. 2, No. 3, 309–319 (1982)

CCC 0198-6325/82/030309-11$02.10

CHEMISCHE BERICHTE

Herausgegeben von der Gesellschaft Deutscher Chemiker

Chem. Ber. **107**, 2284–2294 (1974)

Die Synthese von Purinnucleosid-6-sulfonaten [1]

Hans-Richard Rackwitz und *Karl-Heinz Scheit* *

Max-Planck-Institut für Biophysikalische Chemie, Abteilung Molekulare Biologie, D-3400 Göttingen, Postfach 968

Eingegangen am 19. Februar 1974

6(1*H*)-Purinthion-9-(β-D-ribofuranosid) (**1**), das entsprechende 5′-Monophosphat (**7**) und 5′-Triphosphat (**8**) sowie 2-Amino-6(1*H*)-purinthion-9-(β-D-ribofuranosid) (**2**) und 2-Amino-6(1*H*)-purinthion-9-(β-D-2′-desoxyribofuranosid) (**3**) wurden durch Reaktion mit Sulfit-Ionen in Gegenwart von Sauerstoff quantitativ in die entsprechenden Sulfonsäurederivate **4**, **9**, **10**, **5**, **6** übergeführt. Die Sulfonate **4** und **5** ließen sich bei Raumtemperatur mit wäßrigem Ammoniak in guten Ausbeuten zu Adenosin bzw. 2,6-Diaminopurin-9-(β-D-ribofuranosid) umsetzen. **4** und **9** reagierten mit Aziridin zu 6-(1-Aziridinyl)purin-9-(β-D-ribofuranosid) (**15**) bzw. dem 5′-Monophosphat **16**. **5** und **6** fluoreszieren mit hoher Quantenausbeute im langwelligen ultravioletten Licht. Mit Hilfe der Fluoreszenzspektroskopie, Absorptionsspektroskopie und durch Vergleich mit authentischem Material konnte die Bildung der Purinnucleosid-6-sulfonate **4**, **5** und **6** auch bei der Bestrahlung von **1**, **2** und **3** mit Licht der Wellenlänge 325 nm in Gegenwart von Sauerstoff nachgewiesen werden.

The Synthesis of Purinenucleoside-6-sulfonates [1]

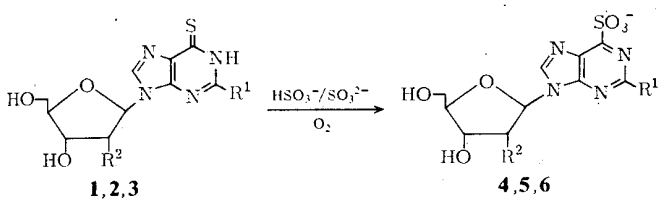
6(1*H*)-Purinethione-9-(β-D-ribofuranoside) (**1**), the corresponding 5′-monophosphate **7**, and the 5′-triphosphate **8** as well as 2-amino-6(1*H*)-purinethione-9-(β-D-ribofuranoside) (**2**) and 2-amino-6(1*H*)-purinethione-9-(β-D-2′-deoxyribofuranoside) (**3**) have been converted to the sulfonates **4**, **9**, **10**, **5**, and **6** by reaction with sulfite ions in the presence of oxygen. The sulfonates **4** and **5** reacted in aqueous ammonia with good yields to give adenosine and 2,6-diaminopurine-9-(β-D-ribofuranoside), respectively. **4** and **9** react with aziridine to form 6-(1-aziridinyl)purine-9-(β-D-ribofuranoside) (**15**) and the corresponding 5′-monophosphate **16**. **5** and **6** fluoresce with high quantum yields on excitation in the near u.v. It was shown by means of fluorescence spectroscopy, absorption spectroscopy, and comparison with authentic material that irradiation of **1**, **2**, and **3** by light of the wavelength 235 nm in the presence of oxygen affords the purinenucleoside-6-sulfonates **4**, **5**, and **6**.

4-Thiopyrimidinnucleoside können sowohl durch chemische Oxidation als auch durch Einwirkung von Sulfit-Ionen in Gegenwart von Sauerstoff in die 2(1*H*)-Pyrimidinon-1-(β-D-ribosid)-4-sulfonate übergeführt werden [2–6]. Diese Verbindungen erwiesen sich als sehr reaktiv gegenüber Nucleophilen. Pyrimidin-4-sulfonate reagieren leicht mit primären oder sekundären Aminen zu den entsprechenden Cytosinderivaten.

[1] Vorgetragen auf dem 24. IUPAC-Kongreß, Hamburg 1973.
[2] *H. Hayatsu* und *T. Ukita*, Biochem. Biophys. Res. Commun. **29**, 556 (1967).
[3] *H. Hayatsu* und *S. Iida*, Tetrahedron Lett. **1969**, 1031.
[4] *H. Hayatsu* und *M. Yano*, Tetrahedron Lett. **1969**, 755.
[5] *E. B. Ziff* und *J. R. Fresco*, J. Amer. Chem. Soc. **90**, 7338 (1968).
[6] *H. Hayatsu*, J. Amer. Chem. Soc. **91**, 5693 (1969).

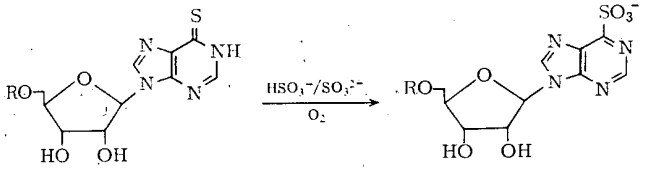
Bei der Bestrahlung von Thiouridin mit Licht der Wellenlänge 330 nm in Gegenwart von Sauerstoff sowie primären bzw. sekundären Aminen wurde ebenfalls die Bildung entsprechender Cytidinderivate in hohen Ausbeuten beobachtet[7,8]. Der Mechanismus dieser Photooxidation ist nicht bekannt, da bisher keine Möglichkeit bestand, die Zwischenprodukte der Photoreaktion zu identifizieren[9]. Es erscheint jedoch wahrscheinlich, daß bei der Photooxidation von 4-Thiouracilderivaten die entsprechenden 2(1*H*)-Pyrimidinon-4-sulfonate als Zwischenstufen durchlaufen werden, aber aufgrund zu großer Labilität nicht nachweisbar waren. Nachdem wir kürzlich diese Photoreaktion für die Affinitätsmarkierung von RNA-Polymerase aus *Escherichia coli* durch 4-Thiouridin-5′-triphosphat und Polydesoxy-4-thiothymidylsäure verwendet hatten[10], war es für uns von Interesse, diese photochemische Oxidation genauer zu untersuchen.

Seit langem war bekannt, daß Purin-6-sulfonate relativ stabil sind[11]. Sollten 6-Thiopurin-Derivate, was anzunehmen war, ebenfalls eine photochemische Oxidation eingehen, so würden die als Zwischenprodukte auftretenden entsprechenden Purin-6-sulfonate mit Sicherheit nachweisbar sein. In dieser Arbeit wird zunächst über die chemische Synthese von Purin-9-(β-D-ribofuranosid)-6-sulfonaten und entsprechender Derivate berichtet. Weiterhin wird gezeigt, daß bei der photochemischen Oxidation von 6-Thiopurinribosiden als Produkte Purin-9-(β-D-ribofuranosid)-6-sulfonate erhalten werden.



1,2,3 → **4,5,6**

	R^1	R^2
1,4	H	OH
2,5	NH_2	OH
3,6	NH_2	H



7,8 → **9,10**

	R
7, 9	$-P(O)(OH)_2$
8,10	$-P(OH)-O-P(OH)-O-P(OH)_2$ (O, OH, OH)

[7] *A. Favre* und *A. M. Michelson*, J. Mol. Biol. **58**, 367 (1971).
[8] *M. Pleiss* und *P. A. Cerutti*, Biochemistry **10**, 3093 (1971).
[9] *M. Pleiss*, *H. Ochiai* und *P. A. Cerutti*, Biochem. Biophys. Res. Commun. **34**, 70 (1969).
[10] *A. M. Frischauf* und *K. H. Scheit*, Biochem. Biophys. Res. Commun. **53**, 1227 (1973).
[11] *I. L. Doerr*, *I. Wempen*, *D. A. Clark* und *J. J. Fox*, J. Org. Chem. **26**, 3401 (1961).

6-Thioinosin (**1**), 6-Thioguanosin (**2**) und 2'-Desoxy-6-thioguanosin (**3**) reagierten mit Sulfit-Ionen in Gegenwart von Sauerstoff quantitativ zu den entsprechenden Purin-9-(β-D-ribosid)-6-sulfonaten **4**, **5** und **6**. Unter den gleichen Bedingungen wurden aus 6-Thioinosin-5'-phosphat (**7**) und 6-Thioinosin-5'-triphosphat (**8**) die entsprechenden Sulfonsäurederivate **9** und **10** erhalten. Die Umwandlung der 6-Thiopurin-Derivate in die entsprechenden Purin-6-sulfonate konnte bequem absorptionsspektrophotometrisch verfolgt werden, da eine starke Abnahme der Absorption bei 322 nm auftrat. Die Verbindungen **5** und **6** zeigten ein Fluoreszenz-Emissions-Spektrum mit einer Quantenausbeute von $Q = 0.47$ (natürliche Abklingdauer der Fluoreszenz 11 ns). Die Identität der Fluoreszenz-Excitationsspektren mit den Absorptionsspektren erlaubte einen eindeutigen Nachweis dieser Verbindungen selbst im Gemisch mit anderen absorbierenden Substanzen.

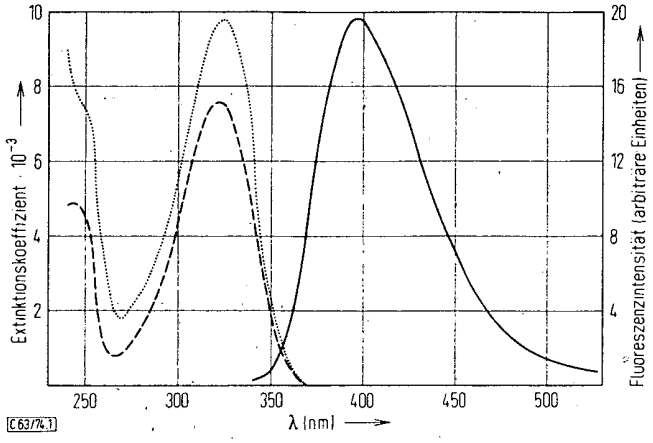
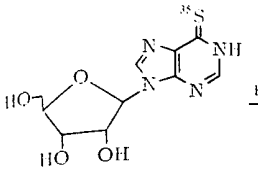


Abb. 1. Spektroskopische Eigenschaften von 2-Aminopurinribosid-6-sulfonat (**5**). (– – –) UV-Spektrum in Wasser pH 6; (· · · · ·) Fluoreszenz-Excitations-Spektrum (Emissionswellenlänge 400 nm); (———) Fluoreszenz-Emissions-Spektrum (Anregungswellenlänge 325 nm)

Die Isolierung der Substanzen **4**, **5**, **6**, **9** und **10** erfolgte durch Anionen-Austauscherchromatographie an DEAE-Cellulose in Ausbeuten von 87–96%. Die Umsetzung von 6(1*H*)-Purin[^{35}S]thion-9-(β-D-ribofuranosid) (**11**) mit Sulfit-Ionen führte zu Purin-6-[^{35}S]sulfonat-9-(β-D-ribofuranosid) (**14**) mit einer spezifischen Radioaktivität von 50% verglichen mit **11**. Die Reaktion kann also nicht nach einem einfachen Additions-Eliminierungsmechanismus verlaufen, da sonst die Verbindung **14** kein ^{35}S mehr enthalten dürfte.

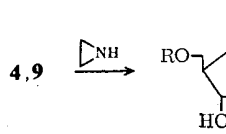
In Analogie zum 4-Thiouridin[6] führt die Addition eines Sulfit-Ions an die Thioxogruppe von **11** zur Bildung einer den Sulfit-Additionsverbindungen von Ketonen analogen Zwischenstufe **12**. Wir nehmen an, daß das gebildete Thioanion **12** zu einer Verbindung **13** oxidiert wird, aus welcher sowohl Sulfit als auch [^{35}S]Sulfit eliminiert werden kann.



11

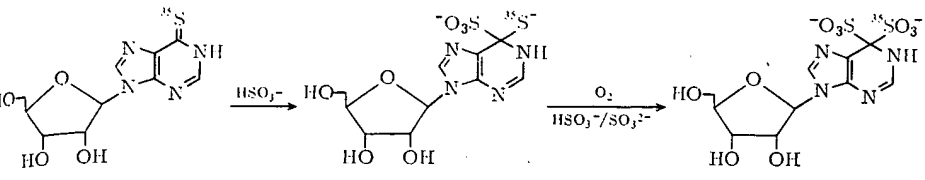
In 0.1 N HCl hydrolysierten d
Raumtemperatur zu Inosin bzw
dünnschichtchromatographisch
von pH 10 setzten sich **4** und
furanosid) um.

Die Purinnucleosid-6-sulfonat
Synthese von C-6-substituierten
hier die Synthese eines Purinnu
Aziridinring substituiert ist. Bei
temperatur entstanden die Verbi
schaften auszeichneten. **15** reagi
methanthiol in Gegenwart von
thio)äthylamino]purin-9-(β-D-rib

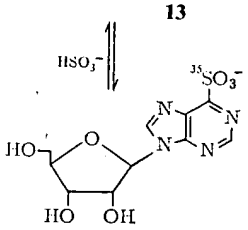


	R
15	H
16	-PO_3H_2

Die Kenntnis der spektroskop
sid-6-sulfonate ermöglichte es u
(**1**), 6-Thioguanosin (**2**) bzw. 2'
wurden in luftgesättigtem Phos
der Wellenlänge 325 nm bestr
sowie **3** absorptionsphotospekt
spektrophotometrisch verfolgt



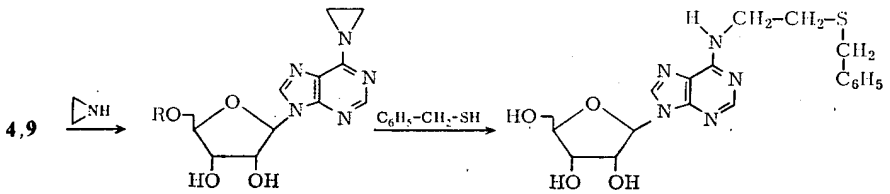
11 **12** **13**



14

In 0.1 N HCl hydrolysierten die Verbindungen **4** und **5** innerhalb von 9 Stunden bei Raumtemperatur zu Inosin bzw. Guanosin, die durch ihre Absorptionsspektren und dünnschichtchromatographisch identifiziert werden konnten. In wäßrigem Ammoniak von pH 10 setzten sich **4** und **5** zu Adenosin bzw. 2,6-Diaminopurin-9-(β-D-ribofuranosid) um.

Die Purinnucleosid-6-sulfonate könnten sich als nützliche Zwischenstufen bei der Synthese von C-6-substituierten Purinnucleosid-Derivaten erweisen. Als Beispiel wird hier die Synthese eines Purinnuclosids gegeben, welches in C-6-Position durch einen Aziridinring substituiert ist. Bei der Reaktion von **4** oder **9** mit Aziridin bei Raumtemperatur entstanden die Verbindungen **15** und **16**, die sich durch alkylierende Eigenschaften auszeichneten. **15** reagierte mit einem hundertfachen Überschuß an Phenylmethanthiol in Gegenwart von Triäthylamin bei Raumtemperatur zu 6-[2-(Benzylthio)äthylamino]purin-9-(β-D-ribofuranosid) (**17**).



	R
15	H
16	$-PO_3H_2$

17

Die Kenntnis der spektroskopischen und chemischen Eigenschaften der Purinnucleosid-6-sulfonate ermöglichte es uns, die Produkte der Photooxidation von 6-Thioinosin (**1**), 6-Thioguanosin (**2**) bzw. 2'-Desoxy-6-thioguanosin (**3**) zu identifizieren. **1**, **2** und **3** wurden in luftgesättigtem Phosphat-Puffer von pH 8.5 bei Raumtemperatur mit Licht der Wellenlänge 325 nm bestrahlt. Der Verlauf der Photoreaktion wurde für **1**, **2** sowie **3** absorptionsphotospektrometrisch, für **2** und **3** darüber hinaus fluoreszenzspektrophotometrisch verfolgt.

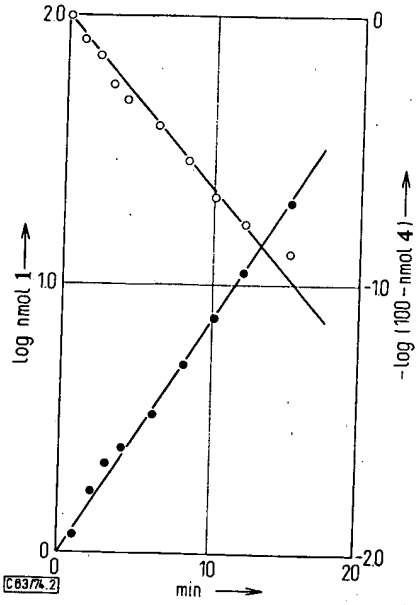


Abb. 2. Kinetik der Photoreaktion von 6-Thioinosin (**1**).
(−●−●) Bildung von **4**; (−o−o) Abnahme von **1**. Reaktionsgeschwindigkeitskonstante $k = 0.08\ \text{min}^{-1}$

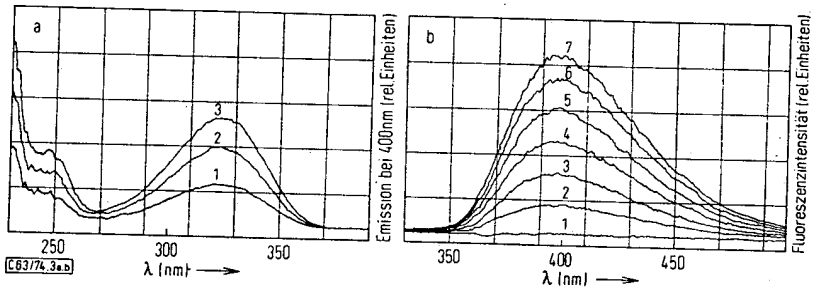
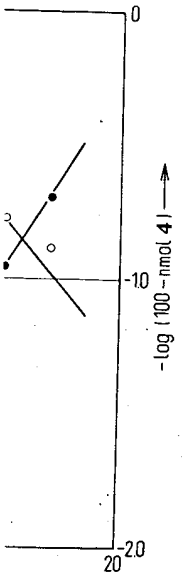
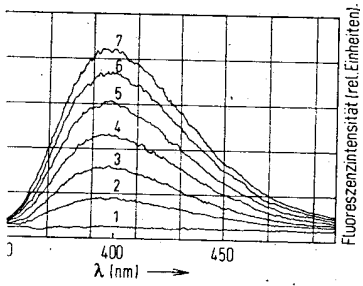


Abb. 3. Photoreaktion von 6-Thioguanosin (**2**).
a) Fluoreszenz-Excitations-Spektren der Reaktionsmischung während der Photoreaktion. Die Spektren wurden in zeitlichen Abständen von 10 min aufgenommen
b) Fluoreszenz-Emissions-Spektren der Reaktionsmischung während der Photoreaktion. Die Spektren wurden in zeitlichen Abständen von 5 min aufgenommen. Anregungswellenlänge 325 nm

Die Absorptionsspektren der Reaktionslösungen ergaben, daß bei der Bestrahlung der 6-Thiopurinnucleoside **1**, **2** und **3** in Gegenwart von Sauerstoff die entsprechenden Sulfonate **4**, **5** sowie **6** gebildet wurden. Bei der Photooxidation von **2** bzw. **3** zeigten die Fluoreszenzspektren der Reaktionsmischungen ebenfalls eindeutig die Bildung eines fluoreszierenden Produkts an, dessen Excitationsspektrum mit dem Absorptionsspektrum von **5** bzw. **6** identisch war. Aus den absorptionsspektrophotometrischen



n von 6-Thioinosin (**1**).
1. Reaktionsgeschwindigkeitskonstante
$]in^{-1}$



Thioguanosin (**2**).
mischung während der Photoreaktion.
len von 10 min aufgenommen
chung während der Photoreaktion. Die
aufgenommen. Anregungswellenlänge

en ergaben, daß bei der Bestrahlung
rt von Sauerstoff die entsprechenden
hotooxidation von **2** bzw. **3** zeigten
en ebenfalls eindeutig die Bildung
ionsspektrum mit dem Absorptions-
absorptionsspektrophotometrischen

Daten ließ sich ein Reaktionsgeschwindigkeitsgesetz pseudo-erster Ordnung für die Bildung von **4** ermitteln. Die Reaktionsgeschwindigkeitskonstante der Photoreaktion beträgt 0.08 min^{-1}.

Experimenteller Teil

Absorptionsspektren wurden mit den Geräten Zeiss PMQ II und Cary 16 aufgenommen. Korrigierte Fluoreszenzspektren wurden mit dem Fluoreszenzspektralphotometer ARL-Fica Modell 55 gemessen. Die natürliche Abklingdauer der Fluoreszenz wurde mit dem nsec-Fluoreszenzspektrometer Modell 9200 in Verbindung mit einem Vielkanalanalysator Modell 6220 der Firma Ortec bestimmt. Schmelzpunkte wurden mit einem Diaskop (Fa. Reichert, Österreich) erhalten und sind nicht korrigiert. Für Photoreaktionen wurde ein He/Cd-Ionenlaser Modell 185 der Firma Spectraphysics (USA) (ca. 2.5 mW/mm² bei 325 nm) benutzt.

Papierchromatographie: Erfolgte absteigend an Papier 2043b gewaschen (Firma Schleicher & Schüll, Deutschland). Für präparative Zwecke wurde Whatman 3 MM Papier verwendet. Lösungsmittel: Isopropylalkohol/konz. NH_4OH/H_2O = 7:1:3 (1), Äthanol/1 M Ammoniumacetat = 5:2 (2), Äthanol/1 M Ammoniumacetat = 1:1 (3).

Dünnschichtchromatographie: Erfolgte an a) DC-Fertigplatten Kieselgel F_{254} (Fa. Merck), b) DC-Mikrokarten Si-F (Fa. Riedel de Haen), c) Polygram CeL 300 PEI/UV_{254}, Polyäthylenimin-imprägnierte Cellulose-Dünnschichtplatten (Fa. Macherey & Nagel).

Lösungsmittel: Chloroform/Methanol = 7:3 (4), Chloroform/Methanol = 85:15 (5), n-Butanol/Wasser = 86:14 (6), Isopropylalkohol/Wasser = 9:1 (7), 0.1 M NaCl (8), 0.25 M NaCl (9), 0.5 M NaCl (10), 1 M NaCl (11).

Papierelektrophorese: Es wurde Papier 2043b gewaschen (Fa. Schleicher & Schüll) verwendet.

Puffer: 0.1 M Borsäure pH 7 (A), 0.05 M Ammoniumformiat pH 3.5 (B), 0.05 M Dikaliumhydrogenphosphat pH 8.5 (C).

6-Thioinosin (**1**) und 6-Thioguanosin (**2**) waren Produkte der Fa. Waldhof (Mannheim, Deutschland).

2'-Desoxy-6-thioguanosin (**3**) wurde von der Fa. PL-Biochemicals (Milwaukee, USA) bezogen. 6-Thioinosin-5'-monophosphat (**7**) und 6-Thioinosin-5'-triphosphat (**8**) wurde nach einer früher berichteten Methode[12,13] hergestellt.

Allgemeine Darstellungsmethode von Derivaten des Purin-6-sulfonats: Zu einer $5 \cdot 10^{-3}$ M wäßrigen Lösung der 6-Thiopurinderivate wurde 1/100 Volumenanteil eines Gemisches von 1 M Na_2SO_3/1 M $NaHSO_3$ = 3:1 (v/v) gegeben. Durch die Mischung wurde Luft gesaugt. Die Zugabe des Sulfit-Gemisches wurde sechsmal in Abständen von 30 min wiederholt. Danach wurde das Reaktionsgemisch zur Trockne eingedampft und der Rückstand mit wasserfreiem DMF extrahiert. Das Eluat wurde eingeengt, der Rückstand in wenig Wasser gelöst und auf eine DEAE-Cellulosesäule gegeben. Die Elution der DEAE-Säule erfolgte mit einem linearen Gradienten an Triäthylammoniumhydrogencarbonat. Fraktionen, welche die gewünschte Substanz enthielten, wurden eingeengt und mit einer 1proz. $NaClO_4$-Lösung in Aceton die Natriumsalze ausgefällt. Die amorphen Niederschläge wurden abzentrifugiert, mit Aceton gewaschen und i. Vak. getrocknet (Tab. 1).

Adenosin: 355 mg (1 mmol) **4** wurden in 10 ml 0.15 M NH_4Cl gelöst und das pH der Lösung mit NH_4OH auf 10 eingestellt. Nach 12 h bei Raumtemp. wurde die Lösung eingedampft und

[12] *P. Faerber* und *K.-H. Scheit*, Chem. Ber. **104**, 456 (1971).
[13] *A. J. Murphy, J. A. Duke* und *L. Stowring*, Arch. Biochem. **137**, 297 (1970).

Tab. 1. Darstellung von Derivaten des Purin-6-sulfonats

Synthese-produkt	Ansatz	Ausb.	Summenformel (Mol.-Masse)	Analyse	UV-Spektrum (λ in nm) Wasser, pH 6.0
4	1.42 g (5 mmol) **1**	1.7 g (96%)	$C_{10}H_{11}N_4O_7SNa$ (354.3)	Ber. C 33.9 H 3.14 N 15.8 S 9.03 Gef. C 34.0 H 3.44 N 15.6 S 8.90	λ_{max} 272 (ε 7.9 · 10³) λ_{min} 229 (ε 1.7 · 10³)
5	748 mg (2.5 mmol) **2**	800 mg (87%)	$C_{10}H_{12}N_5O_7SNa$ (369.3)	Ber. C 32.6 H 3.35 N 19.0 S 8.63 Gef. C 33.0 H 3.76 N 18.5 S 8.70	λ_{max} 322 (ε 7.6 · 10³) 246 (ε 4.8 · 10³) λ_{min} 265 (ε 0.8 · 10³)
6	78 mg (0.25 mmol) **3**	1750 OD-Einh. (322 nm) (92%)	$C_{10}H_{12}N_5O_6SNa$ (353.3)		λ_{max} 322 246 λ_{min} 265
9	26 · 10³ OD-Einh. (322 nm) (1 mmol) **7**	7268 OD-Einh. (272 nm) (92%)	$C_{10}H_{10}N_4O_{10}PSNa_3$ (478.2)	Ber. P 6.48 Gef. P 6.35	λ_{max} 272 (ε 7.8 · 10³) λ_{min} 229 (ε 1.8 · 10³)
10	13 · 10³ OD-Einh. (322 nm) (0.5 mmol) **8**	3412 OD-Einh. (272 nm) (298 mg) (87%)	$C_{10}H_{10}N_4O_{16}P_3SNa_5$ (682.2)	Ber. P 13.6 Gef. P 13.1	λ_{max} 272 (ε 7.8 · 10³) λ_{min} 229 (ε 1.7 · 10³)

der Rückstand der präparativen Schicht unterworfen. Der Inhaltsstoff der am stärk eluiert, das Eluat eingedampft und der 198 mg (74%) farbloser Kristalle vom Sc

UV (Wasser, pH 6): λ_{max} 259 nm (ε 15

$C_{10}H_{13}N_5O_4$ (267.4)

2,6-Diaminopurin-9-(β-D-ribofuranosid) gelöst und mit NH_4OH ein pH von 10 ei temp. belassen. Die ausgefallenen Krista waschen und i. Vak. bei 40°C getrockn Schmp. 250°C (Lit. 240°C[15]).

UV (Wasser, pH 6): λ_{max} 280 nm (ε 10 (Wasser, pH 6): Emissionsmaximum 35 spektrum λ_{max} 280 nm, 255; λ_{min} 265 nm

$C_{10}H_{14}N_6O_4$ (282.3)

6-(1-Aziridinyl)purin-9-(β-D-ribofurano mit 430 mg (10 mmol) Aziridin versetzt gemisch eingedampft, der Rückstand de im Lösungsmittel 4 unterworfen und das Trockne eingedampft, in wenig Methan Niederschlag ausgefällt; Ausb. 172 mg

UV (Wasser, pH 6): λ_{max} 267 nm (ε 1

$C_{12}H_{15}N_5O_4$ (293.

6-[2-(Benzylthio)äthylamino]purin-9-(den in 2 ml wasserfreiem DMF gelöst u Nach Zugabe von 0.2 ml Triäthylamin Die Lösung wurde eingedampft und der an Kieselgel im Lösungsmittel 5 aufgetr eluiert, das Eluat eingedampft und der (63%) farbloser Nadeln vom Schmp. 12

UV (Methanol): λ_{max} 268 nm (ε 16

$C_{19}H_{23}N_5O_4S \cdot C_2H_5OH$ (463.

6-(1-Aziridinyl)purin-9-(β-D-ribofura nm) (0.128 mmol) **9** wurden in 2.5 m Raumtemp. wurde das Reaktionsgemi getrennt. **16** wurde aufgrund seines UV Wasser (1:1, v/v) eluiert, Ausb. 1225

UV (Wasser, pH 6): λ_{max} 267 nm

$C_{12}H_{14}N_5O_7PNa_2$

---

14) *D'Ans-Lax*, Taschenbuch für Che 1964.

15) *K. Imai, A. Nohara* und *M. Honj*

16) *D. C. Ward, E. Reich* und *L. Sty*

der Rückstand der präparativen Schichtchromatographie an Kieselgel im Lösungsmittel 4 unterworfen. Der Inhaltsstoff der am stärksten UV-absorbierenden Zone wurde mit Methanol eluiert, das Eluat eingedampft und der Rückstand aus Wasser umkristallisiert. Es wurden 198 mg (74%) farbloser Kristalle vom Schmp. 230°C (Lit.: 229°C[14]) erhalten.

UV (Wasser, pH 6): λ_{max} 259 nm (ε 15.3·10³), λ_{min} 226 nm.

$C_{10}H_{13}N_5O_4$ (267.4) Ber. N 26.21 Gef. N 25.7

2,6-Diaminopurin-9-(β-D-ribofuranosid): 200 mg (0.54 mmol) **5** wurden in 1 ml Wasser gelöst und mit NH_4OH ein pH von 10 eingestellt. Die Reaktionslösung wurde 3 d bei Raumtemp. belassen. Die ausgefallenen Kristalle wurden abgesaugt, mit wenig kaltem Wasser gewaschen und i. Vak. bei 40°C getrocknet. Ausb. 110 mg (72%) farbloser Kristalle vom Schmp. 250°C (Lit. 240°C[15]).

UV (Wasser, pH 6): λ_{max} 280 nm (ε 10⁴), 255 (ε 9·10³), λ_{min} 265 nm. — Fluoreszenzspektren (Wasser, pH 6): Emissionsmaximum 355 nm (Quantenausbeute $Q = 0.01$)[16]; Excitationsspektrum λ_{max} 280 nm, 255; λ_{min} 265 nm.

$C_{10}H_{14}N_6O_4$ (282.3) Ber. N 29.78 Gef. N 29.10

6-(1-Aziridinyl)purin-9-(β-D-ribofuranosid) (**15**): 354 mg (1 mmol) **4** wurden in 5 ml Wasser mit 430 mg (10 mmol) Aziridin versetzt. Nach 15 h bei Raumtemp. wurde das Reaktionsgemisch eingedampft, der Rückstand der präparativen Schichtchromatographie an Kieselgel im Lösungsmittel 4 unterworfen und das Produkt mit Methanol eluiert. Das Eluat wurde zur Trockne eingedampft, in wenig Methanol aufgenommen und mit Petroläther ein amorpher Niederschlag ausgefällt; Ausb. 172 mg (59%).

UV (Wasser, pH 6): λ_{max} 267 nm (ε 10.4·10³), 275; λ_{min} 229 nm (ε 1.8·10³).

$C_{12}H_{15}N_5O_4$ (293.3) Ber. N 23.9 Gef. N 23.0

6-[2-(Benzylthio)äthylamino]purin-9-(β-D-ribofuranosid) (**17**): 100 mg (0.3 mmol) **15** wurden in 2 ml wasserfreiem DMF gelöst und mit 3.5 ml (30 mmol) Phenylmethanthiol versetzt. Nach Zugabe von 0.2 ml Triäthylamin beließ man das Reaktionsgemisch 12 h bei Raumtemp. Die Lösung wurde eingedampft und der Rückstand durch präparative Schichtchromatographie an Kieselgel im Lösungsmittel 5 aufgetrennt. Das Produkt wurde mit Methanol vom Kieselgel eluiert, das Eluat eingedampft und der Rückstand aus Äthanol umkristallisiert. Ausb. 90 mg (63%) farbloser Nadeln vom Schmp. 123°C.

UV (Methanol): λ_{max} 268 nm (ε 16.5·10³), λ_{min} 223 nm (ε 4.7·10³).

$C_{19}H_{23}N_5O_4S \cdot C_2H_5OH$ (463.6) Ber. C 54.3 H 6.3 N 15.25 S 6.92
Gef. C 53.71 H 6.0 N 15.95 S 7.07

6-(1-Aziridinyl)purin-9-(β-D-ribofuranosid-5-monophosphat) (**16**): 1000 OD-Einheiten (272 nm) (0.128 mmol) **9** wurden in 2.5 ml Wasser mit 0.1 ml Aziridin versetzt. Nach 4 h bei Raumtemp. wurde das Reaktionsgemisch papierchromatographisch im Lösungsmittel 1 aufgetrennt. **16** wurde aufgrund seines UV-Spektrums identifiziert und vom Papier mit Methanol/Wasser (1:1, v/v) eluiert, Ausb. 1225 OD-Einheiten (267 nm) (0.118 mmol, 92%).

UV (Wasser, pH 6): λ_{max} 267 nm (ε 10.4·10³), 275; λ_{min} 229 nm (ε 1.8·10³).

$C_{12}H_{14}N_5O_7PNa_2$ (417.2) Ber. P 7.42 Gef. P 7.84

---

14) *D'Ans-Lax*, Taschenbuch für Chemiker und Physiker, Bd. II, Springer Verlag, Heidelberg 1964.

15) *K. Imai, A. Nohara* und *M. Honjo*, Chem. Pharm. Bull. **14**, 1377 (1966).

16) *D. C. Ward, E. Reich* und *L. Styrer*, J. Biol. Chem. **244**, 1228 (1969).

Tab. 2. Die photochemische Oxidation von Derivaten des 6-Thiopurins (λ in nm)

Synthese-produkt	Ansatz	UV-Spektrum Phosphat-Puffer pH 8.5	Fluoreszenz	Ammonolyse-produkt	UV-Spektrum	A_{250}/A_{260}
4	0.098 μmol **1** in 2 ml Puffer	λ_{max} 272 λ_{min} 229	—	Adenosin	λ_{max} 259 λ_{min} 227	0.82
5	0.1 μmol **2** in 2 ml Puffer	λ_{max} 320, 245 λ_{min} 270	—	2,6-Diaminopurin-9-(β-D-ribofuranosid)	λ_{max} 280, 255 λ_{min} 265	1.0
5	0.045 μmol **2** in 3 ml Puffer	—	Emission λ_{max} 396 Excitation λ_{max} 322, 245 λ_{min} 270	—	—	—
6	0.0187 μmol **3** in 3 ml Puffer	—	Emission λ_{max} 396 Excitation λ_{max} 322, 245 λ_{min} 270	—	—	—

Tab. 3. Papierchromatographie, Dünnschichtchromatographie und Elektrophorese (R_F-Werte)

	Papierchromatographie	Elektrophorese	Dünnschichtchromatographie Kieselgel	Dünnschichtchromatographie PEI-Cellulose

Tab. 3. Papierchromatographie, Dünnschichtchromatographie und Elektrophorese (R_F-Werte)

Substanz	Papierchromatographie			Elektrophorese			Dünnschichtchromatographie							
							Kieselgel					PEI-Cellulose		
	1	2	3	A	B	C	4	5	6	7	8	9	10	11
Adenosin	0.55	0.60	—	1.0	—	—	0.47	0.26	0.30	—	0.62	—	—	—
Guanosin	—	—	—	—	—	—	—	—	—	—	0.64	—	—	—
Inosin	—	0.48	—	—	—	—	0.39	—	—	—	—	—	—	—
1	0.47	0.49	0.69	1.1	—	—	0.51	0.29	0.50	0.73	—	—	0.61	—
2	0.33	0.48	—	0.33	—	—	0.38	0.10	0.46	—	—	0.50	—	—
3	0.41	—	—	0.00	—	—	—	—	—	—	—	—	—	—
4	0.49	0.43	—	2.8	—	0.80	0.00	—	—	—	0.35	0.60	0.77	—
5	0.40	0.30	—	3.0	—	0.66	—	—	—	—	0.24	0.50	—	—
6	0.46	—	—	1.2	—	0.58	—	—	—	—	—	—	—	—
7	—	0.08	0.31	—	1.85	1.06	—	—	—	—	—	—	0.15	0.28
8	—	—	0.18	—	3.48	1.45	—	—	—	—	—	—	—	0.06
9	—	0.07	0.41	—	3.42	1.44	—	—	—	—	—	0.08	0.15	0.44
10	—	—	0.31	—	3.90	1.54	—	—	—	—	—	—	—	0.10
Diaminopurin-nucleosid	—	—	—	—	—	—	—	—	—	0.49	0.45	—	—	—
15	0.70	—	—	—	—	—	0.65	0.51	—	—	—	—	—	—
16	0.44	0.20	—	—	—	—	—	—	—	—	—	—	0.49	—
17	—	—	—	—	—	—	0.73	0.60	—	—	—	—	—	—
AMP	0.29	0.12	—	—	1.00	1.00	—	—	—	—	—	—	0.28	—

Inosin: Eine Probe **4** wurde in 0.1 N HCl gelöst und 9 h bei Raumtemp. belassen. Das pH der Lösung wurde mit 0.1 N NaOH auf pH 6 gebracht und von der Lösung ein Absorptionsspektrum aufgenommen: λ_{max} 248 nm, λ_{min} 223 nm; Verhältnis der Absorptionen 250 nm/260 nm = 1.71, 280 nm/260 nm = 0.23, 290 nm/260 nm = 0.02.

In der Dünnschichtchromatographie an Kieselgel im Lösungsmittel 4 erwies sich das Reaktionsprodukt als einheitlich und identisch mit Inosin.

Guanosin: Eine Probe **5** wurde analog zu **4** hydrolysiert. Dünnschichtchromatographie der Mischung an PEI-Cellulose im Lösungsmittel 8 ergab Guanosin als einziges Reaktionsprodukt.

UV (Wasser, pH 6): λ_{max} 253 nm, λ_{min} 223 nm; Verhältnis der Absorptionen 250 nm/260 nm = 1.15, 280 nm/260 nm = 0.66, 290 nm/260 nm = 0.25.

6-[^{35}S]Thioinosin (**11**): 14.9 mg (0.05 mmol) **1** wurden in 1 ml einer $0.2 \cdot 10^{-3}$ M Lösung von Schwefel in DMF bei 60°C gelöst. Dazu gab man 5 µl 2 M Triäthylammoniumhydrogencarbonat-Puffer pH 7.5, sowie 10 µl einer Lösung von ^{35}S in Benzol (0.026 mCi/µl) und hielt das Gemisch 2 h bei 60°C. Die Lösung wurde auf ca. 0.2 ml eingeengt, von ausgefallenem Schwefel abzentrifugiert und der Überstand an Kieselgel dünnschichtchromatographisch zunächst im Lösungsmittel 5 und anschließend im Lösungsmittel 7 getrennt. Das Produkt wurde vom Kieselgel mit Methanol/Wasser (1:1) eluiert und das Eluat eingedampft. Man erhielt 580 OD-Einheiten (320 nm) (48%) 6-[^{35}S]Thioinosin mit einer spezif. Radioaktivität von 0.5 mCi/mmol.

UV (Wasser, pH 6): λ_{max} 322 nm, λ_{min} 250 nm.

Durchführung der photochemischen Oxidationen: Alle Reaktionen wurden in einer Quarzküvette (Volumen 3 ml, Schichtdicke 1 cm) in 0.1 M K_2HPO_4-Puffer pH 8.5, der mit Luftsauerstoff gesättigt war, durchgeführt. Die Reaktionslösungen wurden bei 25°C in der Küvette mit Licht der Wellenlänge 325 nm bestrahlt. Zu bestimmten Zeiten wurde die Bestrahlung unterbrochen und vom Reaktionsgemisch Absorptions- bzw. Fluoreszenzspektren aufgenommen, sowie Proben für die Dünnschichtchromatographie entnommen. Nach beendeter Photooxidation wurde zu den Reaktionslösungen 0.1 ml konz. NH_4OH hinzugegeben und die stattfindende Ammonolyse absorptionsspektrophotometrisch verfolgt (Tab. 2).

[63/74]

Über Aminosäuren und

Synthese von 2,5-Cy
alanin) über 2,5-Cyc

Dieter Scholz und *Ulrich*

Organisch-Chemisches Institu

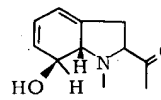
Eingegangen am 8. Februar

2,5-Cyclohexadien-1-acetalde
chlorid in guter Ausbeute he
dien-1-alanin-methylamid (2)
amid (1,4-Dihydrophenylalan

On Amino Acids and Peptides
Synthesis of 2,5-Cyclohexadie
2,5-Cyclohexadien-1-ylmethyl

2,5-Cyclohexadiene-1-acetalde
ylmethylmagnesium chloride
cyclohexadiene-1-alanine met
1-alanine methylamide (1,4-di

Aus der Struktur des Gli
auf deren Biosynthese übe
A und B). Durch Versuche
und Aranotin bewiesen. Ei
brachte die Beobachtung
Gliotoxinaufbau herangezo



Die Bildung des Tetra
Modellreaktion untersucht
alanin (2,5-Cyclohexadien-1
lich die L-2,5-Dihydroverbi
Phenylalanin gewonnen[4] u

[1] IX. Mitteil.: *J. Häusler* un
[2] *N. Neuss, R. Nagarajan,* B
Antimicrob. Ag. Chemoth
[3] *D. R. Brannon,* Biochem.
[4] *M. L. Snow, C. Laninger* u
[5] *G. R. Nagarajan, L. Diam*
Lit. 2–5.